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CLAIMS

Claim(s)

numbers 1 or 2, and its descendant animal, and is characterized by holding the above-mentioned DNA fragment in a the totipotency cell which introduced the DNA fragment including a promotor array and the DNA array of the array [Claim 1] The excess ovulation animal which are the transgenic nonhuman animal which generated to the individual somatic cell chromosome. [Claim 2] The excess ovulation animal of claim 1 whose promotor array is a promotor array of the receptor gene of a gonadotropic hormone.

[Claim 3] The excess ovulation approach characterized by making the protein which is the approach of promoting [Claim 4] The excess ovulation approach characterized by making the protein which is the approach of promoting artificially ovulation of an excess ovulation animal according to claim 2, medicates an animal with a gonadotropic controlling factor of a promotor array, and has the amino acid sequence of the array numbers 3 or 4 discover. artificially ovulation of an excess ovulation animal according to claim 1, medicates an animal with the imprint hormone, and has the amino acid sequence of the array numbers 3 or 4 discover.

[Claim 5] The excess ovulation animal introduced into the ovary in the oocyte which carried out the transformation by the recombination vector containing a DNA fragment including a promotor array and the DNA array of the array

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numbers 1 or 2.

[Claim 6] The excess ovulation animal of claim 5 whose promotor array is a promotor array of the receptor gene of gonadotropic hormone.

[Claim 7] The excess ovulation approach characterized by making the protein which is the approach of promoting controlling factor of a promotor array, and has the amino acid sequence of the array numbers 3 or 4 discover. artificially ovulation of an excess ovulation animal according to claim 5, medicates an animal with the imprint

Claim 8] The excess ovulation approach characterized by making the protein which is the approach of promoting artificially ovulation of an excess ovulation animal according to claim 6, medicates an animal with a gonadotropic hormone, and has the amino acid sequence of the array numbers 3 or 4 discover.

[Claim 10] The recombination vector containing a DNA fragment including a promotor array and the DNA array of the artificially, and is characterized by making the protein which has the amino acid sequence of the array numbers 3 or by medicating an animal individual with protein kinase repressor, SUTAUROSUPORIN, or those derivatives discover. [Claim 9] The excess ovulation approach which is an approach of promoting ovulation of a naive animal individual array number 1. [Claim 11] The recombination vector containing a DNA fragment including the promotor array of the receptor gene of a gonadotropic hormone, and the DNA array of the array number 1

[Claim 12] The recombination vector containing a DNA fragment including a promotor array and the DNA array of the array number 2.

[Claim 13] The recombination vector containing a DNA fragment including the promotor array of the receptor gene of a gonadotropic hormone, and the DNA array of the array number 2.

[Claim 14] The cell isolated from the excess ovulation animal according to claim 1 or 2.

[Claim 15] The cell of claim 14 whose cell is a reproductive cell.

[Claim 16] Oocyte which carried out the transformation by the recombination vector containing a DNA fragment ncluding a promotor array and the DNA array of the array numbers 1 or 2.

Claim 17] Oocyte of claim 16 whose promotor array is a promotor array of the receptor gene of a gonadotropic

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DETAILED DESCRIPTION

Detailed Description of the Invention]

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transgenics excess ovulation animal to which the number of ovulation of a mature ovum can be made to increase, and Field of the Invention] This invention relates to the approach of controlling artificially the number of ovulation of the a this excess ovulation animal or a naive animal.

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primordial follicles reach an ovulation process as the number of the primordial follicles at the time of birth is restricted ovary of the female individual of mammalian. This primordial follicle is what consists of oocyte which is a reproductive [Description of the Prior Art] Many primordial follicles (primodialfollicles) are formed in fetus or after the birth in the cell which will become an ovum in the future, and a granulosa cell which surround it. The number decided for every vesicularfollicles), And it grows to the Graafian follicle (graafian follicles), finally oocyte matures, and it is ovulation by animal species and shown in <u>drawing 1</u> , and a part finishes with hibernation. A primordial follicle is the "atresia sexual cycle through the whole life of an individual these primordial follicles The primary ovarian follicle (primary (ovulation). The process in which it results is stepped on. However, it is 99.9% although it is only that very few follicles), The secondary follicle (secondary follicles) and the vesicular ovarian follicle (antral follicles or

folliculi" (atresia) at the growth way. It backs through the process said.

p53 grade, ced-3/interleukin 1 converting enzyme (ICE:) interleukin-1 beta converting enzyme Related gene (Flaws et nutrition, ischemia, etc. were mentioned as a factor whose ovarian follicle backs, the detailed molecule mechanism had 1994; Tilly et al. and Endocrinology 136: 1394-14023, 1995; Tilly and Tilly and Endocrinology 136: 242-252 and 1995, A Endocrinol.6 : 1942–1950, 1992;Chun et al. and Endocrinology 135: 1845–1853, 1994; Tilly et al. and Endocrinology 136: morphological and biochemical and histological change, concentration of nucleus chromatin and nuclear fragmentation et al. and "reference name --) A magazine name, vol.page 1992; Chun" et al. and Endocrinology 135 : 1845-1853 and are accepted in the granulosa cell of an atretic follicle, and closing of ovarian follicle and the relation of apotosis are suggested. from bacterial research, a gonadotropic hormone controls the apotosis in the atretic follicle of a rat (Tilly the optional feature of ovarian follicle The active oxygen in the granulosa cell depending on a gonadotropic hormone 1394–14023 and 1995 are clear. furthermore, as backing of possibility that apotosis serves as an important point of (Tilly and Tilly, Endocrinology 136:242–252, 1995), The antioncogene (Tilly et al., Endocrinology 136:1394–14023) of [0003] Although age, a breeding cycle, pregnancy, lactation, the hormone balance of ovary inside and outside, a part of the depressant action moreover, the growth hormone in ovarian follicle It minds (). [Tilly et al.,] [Mol.] been unknown for a long time. in recent years, it sees in the case of the atresia folliculi –– from observation of al., Endocrinology 136:5042-5053) Change is reported.

although it was reported very much recently that the Bcl-2 related gene product which controls apotosis and has the 136:3665- 1995). Possibility that apotosis repressor different from a Bcl-2 related gene product is involving from this suggested that Bcl-2 are participating only in the survivability of a primordial follicle (3668 Ratts et al., Endocrinology [0005] On the other hand, in connection with development of the molecule biological technique in recent years, it is prolongation-of-life function of a cell is discovered by the ovarian follicle in the rat ovary (Tilly et al., Endocrinology [0004] However, the detailed elucidation was not made about what kind of function is achieved in the device which controls survival and selection of the ovarian follicle in the ovary containing the oocyte these factors of whose are 36:232-241, 1995) From observation of the mouse which made Bcl-2 gene suffer a loss by gene targetting, it was reproductive cells, or whether the factor which controls apotosis further is related to how [those]. however in process of degradation and closing of the primary ovarian follicle which grew from the primordial follicle, the secondary follicle, and the ovarian follicle in the ovary in the vesicular ovarian follicle is assumed

introduced into various cultured cells, and when **** to which induction of appointment **-SHISU is carried out was muscular-atrophy syndrome (spinalmuscular atropy: SMA) which is a familial hereditary disease by positional cloning mechanism of the apotosis which cannot be explained only by the intervention of a Bcl-2 related gene product was which is - ** of the technique. It was isolated (Royet al., Cell 80:167-178, 1995). Furthermore, this NAIP gene was nerve cell apotosis control protein (nural apoptotic inhibitory protein:NAIP) as a gene of cause of the spine nature given to the cell, it became clear that that cell death is controlled (Liston et al., Nature 379:349–353, 1996). From these results, possibility that NAIP was a factor which has a cell prolongation-of-life-function in the controlling

device which eliminates an unnecessary cell when maintaining a living body's homeostasis which consists of countless programmed by the animal species. Therefore, if it becomes possible to control this NAIP gene expression artificially, only controls the apotosis of a nerve cell, but is considered to function as keeping constant the number of ovulation cells. The manifestation of NAIP in an animal individual controls degradation and closing of ovarian follicle, and it not the number of ovulation will become possible [producing efficiently the useful animals (livestock animals, such as a cow and a horse etc.) which are fractions] in spite of the ovulation inducing drug processings including the sterility [0006] In in the living body, the burying-him-alive-cell death called apotosis is a phenomenon indispensable as a therapy in Homo sapiens. [0007] This invention is made in view of the situation as above, and aims at offering the excess ovulation animal which holds all the cDNA arrays of a foreignness NAIP gene. Moreover, this invention aims at offering the approach of promoting the number of ovulation of the above-mentioned animal artificially.

ovulation of a naive animal individual (animal individual into which the foreignness gene is not introduced) including [0008] Furthermore, this invention is required for the purpose of offering the approach to which the number of Homo sapiens is made to increase artificially. Means for Solving the Problem] This invention is the transgenic nonhuman animal which generated to the individual the totipotency cell which introduced the DNA fragment including a promotor array and the DNA array of the array numbers 1 or 2 as what solves the above-mentioned technical problem, and its descendant animal, and offers the excess ovulation animal (claim 1) characterized by holding the above-mentioned DNA fragment in a somatic cell

chromosome.

[0010] Moreover, this invention offers the excess ovulation animal (claim 5) introduced into the ovary in the oocyte which carried out the transformation by the recombination vector containing a DNA fragment including a promotor above-mentioned promotor array makes it the desirable mode to be the promotor array of the receptor gene of array and the DNA array of the array numbers 1 or 2. In addition, in these excess ovulation animals, the gonadotropic hormone (claims 2 and 6).

sequence of the array numbers 3 or 4 discover. A promotor array is the approach of promoting artificially ovulation of [0011] Furthermore, this invention is the approach of promoting artificially ovulation of the above-mentioned excess offers the excess ovulation approach (claims 3 and 7) characterized by making the protein which has the amino acid ovulation animal (claims 1 and 5), medicates an animal with the imprint controlling factor of a promotor array, and the excess ovulation animal (claims 2 and 6) which is the promotor array of the receptor gene of a gonadotropic hormone, and this invention medicates an animal with a gonadotropic hormone, and offers the excess ovulation approach (claims 4 and 8) characterized by making the protein which has the amino acid sequence of the array numbers 3 or 4 discover further again.

[0012] Furthermore, this invention is the approach of promoting ovulation of a naive animal individual artificially, and sequence of the array numbers 3 or 4 discover by medicating an animal individual with protein kinase repressor, offers the excess ovulation approach (claim 9) characterized by making the protein which has the amino acid SUTAUROSUPORIN, or those derivatives.

the DNA array of the array numbers 1 or 2 (claims 11 and 13), The cell (claim 14) isolated from the above-mentioned vector containing a DNA fragment including the promotor array of the receptor gene of a gonadotropic hormone, and [0013] The recombination vector containing the DNA fragment with which this invention besides the above invention transgenic nonhuman animal and the oocyte (claim 16) which carried out the transformation by the recombination vector containing a DNA fragment including a promotor array and the DNA array of the array numbers 1 or 2 are includes a promotor array and the DNA array of the array numbers 1 or 2 (claims 10 and 12), The recombination offered, respectively.

[0014] Hereafter, the gestalt of implementation of this invention is explained in detail.

field (5q13.1), and that overall length cDNA has the base sequence of the array number 1 or the array number 2. such Embodiment of the Invention] In this invention, the gene used as a means of degradation / closing control of ovarian overall length of the DNA array of two is sufficient as cDNA introduced into an animal individual, or the coding region follicle is a NAIP gene isolated as a gene of cause of SMA from 5th chromosome macrobrachia of Homo sapiens 13.1 part is sufficient as it. And in creation of the excess ovulation animal of this invention, this cDNA array and the DNA luteal hormone, is desirable especially. Or the promotor array of the foreign gene which uses the matter which is not the promotor array of the receptor gene of gonadotropic hormones, such as follicle-stimulating hormone and corpus [0016] Since a promotor array makes this cDNA array discover within the ovary, it is desirable to use the promotor array of the gene which uses as an imprint controlling factor the matter which exists in an ovary unique target, and ibrary of the various existing animal origins -- it can isolate -- or a part of array numbers 1 or 2 -- by making an inherent in the animal species to be used as an imprint controlling factor can also be used. In this case, it can be array into a primer, PCR magnification can be carried out and it can obtain. Moreover, the array number 1 or the cDNA $^{--}$ for example, 1 section array of the array numbers 1 or 2 $^{--}$ a probe $^{--}$ carrying out $^{--}$ from the cDNA fragment which connected the promotor array with that upstream are prepared, and the transgenic animal which generated to the individual the totipotency cell which introduced this DNA fragment is created.

frame is made in agreement, a promotor array and the DNA array (or that part array) of the array numbers 1 or 2 are [0017] For example, the transgenic animal of this invention can be created by the following approaches. Each reading which poured in the DNA fragment, the animal generated to the individual is made born, and it breeds. And a somatic calcium phosphate method, a microinjection method, etc. Next, transplant to the oviduct of assumed parents the cell dog, and a cat, the primates, an animal for an experiment, etc. Moreover, as a totipotency cell, a cultured cell like an cell is taken out and existence of the DNA fragment which carried out Southern blot analysis of the DNA in this cell, ****(ed), a DNA fragment is prepared, and this DNA fragment is introduced into the totipotency cell of a nonhuman these totipotency cells can use a well-known approach, i.e., an electrostatic pulse method, the liposome method, a mammal. The target animals are useful livestock, such as a cow, Buta, a horse, and a sheep, or are pets, such as a embryonic stem cell besides a fertilized egg or an early embryo can be used. Impregnation of the DNA fragment to

made discovered, only when introduced cDNA is not spontaneously discovered and prescribes that imprint controlling

actor for the patient from the exterior.

fragment was checked. (Founder) Then, this introductory DNA fragment is transmitted to 50% of that descendant, and and introduced it is checked. He is the founder about the individual by which inclusion for the chromosome of a DNA can produce the excess ovulation animal of this invention continuously.

exists, and cDNA controls degradation and closing of ovarian follicle. Of course, although the NAIP gene of internality follicle-stimulating hormone, it is specifically discovered only by the granulosa cell of the ovary in which the hormone introduced foreignness, compared with a naive animal, a lot of ovulation is possible for it. Moreover, excess ovulation above-mentioned DNA fragment directly in the ovary of an animal individual. That is, the recombination vectors (an adenovirus vector, retrovirus vector, etc.) incorporating a DNA fragment are transplanted to the ovary of an animal [0018] And the excess ovulation animal which carried out in this way and was created has the introductory DNA existence of a specific imprint controlling factor. When a promotor array is the promotor of the receptor gene of invention discovers degradation / closing repressor of ovarian follicle so much with the NAIP gene cDNA of the exists in an animal individual and the number of ovulation is controlled, since the excess ovulation animal of this [0019] On the other hand, the excess ovulation animal of this invention can be created also by introducing the fragment for the chromosome of all the cells, and makes a promotor array discover cDNA of a NAIP gene by is also artificially controllable by medicating a promotor with a specific imprint controlling factor.

an animal rather than a naive animal into the ovary, and it serves as excess ovulation. Moreover, it is possible to control the excess ovulation by prescribing for the patient the imprint controlling factor of the promotor array connected with the NAIP gene cDNA.

recombination vector is transplanted to the ovary. A NAIP gene carries out the abundant manifestation also of such

individual by the physical approach. Or the oocyte which carried out the transformation by the above-mentioned

lot of ovulation is attained in an animal individual including Homo sapiens, without using an ovulation inducing drug etc., artificially further again. That is, all animal individuals are equipped with the NAIP gene into that genome, and the gene repressor (for example, immunosuppresant FK506 grade), or SUTAUROSUPORIN (K252A etc.) or these derivatives. A [0020] This invention also offers the excess ovulation approach of promoting ovulation of a naive animal individual of this internality can be made to discover transient by carrying out whole body administration of protein kinase and insurance and a positive sterility therapy are offered by this.

[0021] Next, the experimental result which checked that a NAIP gene was a gene which surely participates in

from Japanese Clare, Inc.) was carried out for ** term 12 hours (5:00 - 17:00) at the time of 3 weeks old. That is, the an ingredient, an approach animal, and the gonadotropic hormone processing ICR system female mouse (it purchases (1) Humidity and temperature were bred under the environment adjusted uniformly, and superovulation processing of intraperitoneally, and the Homo sapiens chorionic gonadotropin (human chorionic gonadotropin, hCG) of 5IU was pregnant-mare-serum gonadotropic hormone (pregnant mare serum gonadotropin, PMSG) of 5IU was injected degradation / closing control of ovarian follicle is shown, and the effectiveness of this invention is explained. injected intraperitoneally similarly 48 hours after.

carried out silane coat *** after thin sectioning at the intercept. After making it dry, the organization intercept which after being immersed in the fresh paraformaldehyde of 4 % for 5 minutes and making it re-fix to it, it processed in the 0.2 % glycine for 1 hour, residual ARUDEHITO was neutralized, and it ****(ed) by formamide 50% as pre-hybridization Protease (Triton–X (2 between parts), 0.2 %HCI (for 20 minutes), and 20microg/ml) (for 20 minutes) K. Furthermore, hybridization BUAN immobilization, and it is 5-6 micrometers in thickness. They are 10 ** to the slide glass which performed deparaffinization row hydrophilic actuation according to the conventional method was ***(ed) 0.3% by in Carry out paraffin embedding after carrying out dehydration *** of the mouse ovary which carried out situ

condition in the moisture chamber for 16 to 20 hours using 65 degrees C and the solution which added the RNA probe [0022] In antisense one of NAIP and the sense RNA probe which are used for high buri die ZEJON Homo sapiens naip gene (Roy et al. --) Cell 80:167-178 and 195 of 1995 A part of BIR1, BIR2, and BIR3 of the BIR (baculoviral inhibition fragment (<u>drawing 2</u>). – The vector which carried out NINGU is used. Digoxigenin (Digoxigenin:DIG)-RNA labeling Kit preparation, the following washing actuation was performed. First, after washing for 20 minutes in 4XSSC, processing imprint. In addition, about this PU opening 1 BU, the gay opening G with mouse c-IAP1 which is an IAP (inhibitor of denatured by the processing for 5 minutes beforehand. Next, in order to remove an unreacted RNA probe from the salmon sperm DNA, 100microg [/ml] Escherichia coli tRNA, and 10 mM DICHIOSUTE oar under the 50-degree C formamide, 10% dextran sulfate, 1X Denhardt's solution, and 100. It carried out to ***** of mug/ml single strand of apoptosis protein repeat) field from a base to 1263 bases It is a subclo to pBluscript about the included gene of Boehringer Mannheim The generated DIG indicator RNA probe was used by using and carrying out an in vitro apoptosis protein) related gene is 30-40%, and is understood that crossover nature is low. Hybridization is 50%

for 30 minutes and washing 37 degrees C in 20microg [/ml] RNase, it reaches 2 XSSC under a 68 more-degree C condition. It washed in 0.2XSSC for 1 hour each.

Laboratories) fundamentally, carrying out PU opening TEAZE processing of the organization intercept first and making [0023] 5-bromo-4-chloro-3-indoyl-phosphate-nitroblue tetrazolium after making the alkali FOSUFATA 1 ZE indicator (TUNEL) whose detection of morphological apotosis is the approach of detecting fragmentation in the NUKURESOMU signal in which existence of RNA of a NAIP gene is shown according to considering as a substrate and making it color under 4-degree C conditions was detected. About creation of BUAN immobilization of the histochemistry-detection DIG antibody of Boehringer Mannheim react in detection by the immunohistochemistry reaction (BCIP/NBT) The approach correspondingly. Terminal Deoxynucleotidly Transferase-(TdT) mediated dUTP-biotin nick end labeling mouse ovary of apotosis, paraffin embedding, and an organization intercept, it applied to the above-mentioned unit of Chromosome DNA in histochemistry –– law was used. After using MEBSTAIN Kit (Medical & Biological streptoavidin-HRP (horseradish peroxidase), combined TUNEL assay, it was made to color using DAB the amount of [of the fragmentation DNA in a nucleus] 3'OH end incorporate biotin-ized dUTP, add (diaminobenzidine) as a substrate, and Fragmentation DNA was detected to it.

The ovary is extracted from the ICR juvenile female mouse of isolation of a granulosa cell, and 3 weeks old culture, fat an RNA extract and the extract of all RNA from reverse transcriptase PCR (RT-PCR) and the northern-blot-analysis initiation, the cell lump of the granulosa cell containing these oocytes was moved to the MEM containing 100 ng(s)/ml containing oocyte of a granulosa cell — Eppig ** — (Biol.Reprod., 41:268–276, 1989) Hirano and others (J.Exp.Zool., lump which isolated did the coat with the agar -- and -- It cultivated by 2mM hypoxanthine. Ten days after culture ollicle-stimulating hormone (fillicle-stimulating hormone: FSH and sigma), the cell lump of the granulosa cell which 267:543-547, 1993) It carried out almost according to the reported approach. First, it was immersed and the ovary tissue, blood, etc. are removed on a filter paper, and it is an Eagle's minimum essential medium (minimum essential extracted to the MEM which added 2mg [/ml] collagenase (the object for cell distribution, Wako Pure Chem) was granulocyte containing oocyte was isolated. 4mg [/ml] bovine serum albumin in the culture plate in which the cell processed for 30 minutes. Pipetting was repeated with Pasteur pipette after washing, and the cell lump of the medium:MEM). It offered as a sample to the experiment after washing. the isolation and culture of a cell lump contains oocyte 18 hours after was extracted, and the analysis of NAIP gene expression was presented.

ovary --- AGPC (acid guanidium thiocyanate-phenol-chloroform) -- law (Anal.Biochem.162:156-159, 1987) It carried out. After all extracted RNA digested the genomic DNA mixed by carrying out DNaseI processing, the quantum of it was carried out and the experiment was presented with it.

reaction condition carried out 94 degrees C of thermal denaturation for 5 minutes, it wound annealing 60 degree C for -CACAGGGGTGAAACTTGGGGTTCAG-3' 5 [and] --- '-CACCTGTGGTTTCCATGGCTTCTGG-3' it is -- after the 94 degrees C of thermal denaturation for 1 minute, wound the cycle for 2 minutes for 1 minute and for 72 degrees C (TAKARA SHUZO), and cDNA of Mouse naip was amplified using the part. the primer used for magnification -- 5 ---[0024] By RT-PCR analysis, it is 1microg. From all RNA to AMVXL Therefore, cDNA was compounded to RTase of DNA synthesis 40 times, and removed. Electrophoresis of the amplified RT-PCR product was carried out by agarose gel 2%, and it was separated and detected.

(Pori A) +RNA was used. It is a nylon filter after separating agarose electrophoresis under existence of a formamide. It First, the mouse nnaipcDNA probe which carried out 32 P-dCTP indicators was added after the pre hybridization of 2 the water solution of 2XSSC and 0.05%SDS 68 degrees C and 20 Between parts was performed by carrying out twice. transferred to ybondN+ (Amersham). UV irradiation was carried out to the dried nylon filter on the next day, and RNA BIR2 and BIR3 It is the gene fragment of the die length of a base ($\overline{drawing~2}$). Too, the homology with mouse c-IAP1 which is an IAP related gene is 30 – 40%, and is understood that crossover nature is low. Washing of hybridization is [[0025] 6-8microg refined in NO 1 Zhang blotting analysis using Oligotex-dT<Super> (TAKARA SHUZO) from all RNA a room temperature and] for 20 minutes in the water solution of O.1XSSC and 0.1 %DSD to 2 times and a degree in mouse naip gene (Robertson et al., unpublished data). 854 containing a part of BIR1 from a base to 1326 bases, and 3 hours, and hybridization was performed overnight. In addition, the gene fragment used for the probe is 473 of a on a filter was fixed. ExressHyb hybridization solution (Clonetec) was used in pre hybridization *** hybridization. The washed filter performed autoradiography by the imaging plate (FUJI), detected the signal by BAS-2000 and performed quantitative analysis.

ovary extracted from the female mouse of the weeks old of NAIP gene expression versatility accompanying growth of (2) in situ hybridization using a specific RIBOPU lobe [as opposed to the cDNA for the NAIP gene expression in the the ovarian follicle in the result ovary] By investigating, the NAIP gene expression accompanying growth of the ovarian follicle in the ovary was examined.

were two ovarian follicle images of the primordial follicle by which oocyte was surrounded by much more flat granulosa [0026] Although the ovarian follicle images in the ovary observed in 2 age-in-day mouse ovary immediately after birth cell or the granulosa cell which became cube-like, oocyte, and the primary ovarian follicle which consists of basement weeks old which repeats sexual cycle regularly matured, NAIP gene expression was accepted also in the cumulus cell antisense RIBOPU lobe in the granulosa cell of the primary ovarian follicle which has the oocyte which grew more on ovulation (drawing 3 (B)). [0027] From the above result, having discovered the NAIP gene by the granulosa cell from the other hand (drawing 3 (A)). Next, when NAIP gene expression was investigated in the mouse ovary in which 12 which surround the granulosa cell and oocyte of a Graafian follicle which are vesicular ovarian follicle in front of membrane of a periphery, in a primordial follicle, a difference was not regarded as the case where sense and an antisense RIBOPU lobe are made to hybridize, either. The strong signal was observed by the time of using an the primary ovarian follicle to the Graafian follicle in front of ovulation was checked.

NO 1 Zhang blotting analysis investigated the NAIP gene expression in each organization of the mouse containing the tissue-specific-expression ovary of a NAIP gene by using Mouse naipcDNA as a probe.

ovary of the 2 age in day and the day [of a delivery / 3rd] female mouse with which a primordial follicle and a corpus weeks old, and the 3rd day of a delivery And 8microg of the ovary origin extracted from the various female mice of 18 blot, Clonetec) which combined (A)+RNA, there is no tissue specificity and the manifestation was mostly accepted in all organizations (drawing 4 (A)). On the other hand about a manifestation in the ovary, 2 age in day, 3 weeks old, 12 gene expression in the main organizations of a mouse was examined using mouse multiple-tissue northernblot (MTN expression was observed with the female mouse of 12 weeks old and 18 weeks old which repeats sexual cycle with ovary. First, 2microg Pori Although the strong signal was seen with ***, lungs, liver, and the heart when the NAIP weeks old Pori The place which used the filter which carried out BUROTSU ** of the (A)+RNA, Strong NAIP gene many rates of primary, the secondary follicle, or the vesicular ovarian follicle and which matured. However, in the [0028] The NAIP gene is discovered as a transcript with two die length in mouse each organization including the uteum occupy many, respectively, NAIP gene expression was weak.

[0029] From the above thing, it was checked that the NAIP gene expression in the ovary is in the development process and correlation of ovarian follicle.

The localization gonadotropic hormone of the NAIP gene expression in ovarian follicle investigated the manifestation

before -- comparing -- about 2.4 twice -- the strong manifestation was observed. When RT-PCR detected the NAIP although there was no absolute quantum nature for magnification by PCR, the inclination for a NAIP gene expression gene expression in the cell lump of the granulosa cell which contains next the oocyte isolated from the ovary, it was amount of NAIP gene expression in the ovary 48 hours [with the operation as follicle-stimulating hormone (FSH)] shown by oocyte that the manifestation is discovered only by private seal ** and the granulosa cell. Furthermore, after PMSG administration -- before administration -- comparing -- about 1.6 It is twice and has a corpus luteal hormone (Lutinating hormone, LH) operation further. administration hours [7 hours] after hCG -- administration of a NAIP gene with time in the ovary of the female mouse of 3 weeks old which gave superovulation ***. the signal to become strong with a gonadotropic hormone was observed.

observed in the granulosa cell of the ovarian follicle which the NAIP gene has discovered strongly. On the other hand, observed, and by such ovarian follicle, NAIP gene expression is feeble or was hardly observed. [0031] Since the NAIP [0030] It was checked that carry out localization of the NAIP gene expression to the granulosa cell of ovarian follicle, gene was not discovered by the closed ovarian follicle as above, it was checked that the NAIP gene is functioning as expression detected and the TUNEL assay was carried out. Consequently, as shown in <u>drawing 5</u> , apotosis was not by the atretic follicle characterized by deformation of oocyte, the granulosa cell which carried out cell death was expression, and is in situ hybridization. Comparison examination of the apotosis accepted by the NAIP gene It sets on the continuation organization intercept of the related ovary of the atresia folliculi and NAIP gene and the manifestation is reinforced with gonadotropic hormones, such as FSH, from the above result. apotosis repressor in the ovarian follicle in the ovary.

[0032]

degradation and closing of ovarian follicle as explained in detail above. By this, development of a new sterility therapy expression by this invention with the excess ovulation animal which introduced the NAIP gene which controls Effect of the Invention] The approach of promoting ovulation artificially is offered by controlling NAIP gene can be attained and the productivity of a useful animal can also be raised

[0033

[Layout Table]

array number: -- die-length [of one array]: -- mold [of 5984 arrays]: -- number [of nucleic-acid chains]: --

GGAATCAGCT GTGGGAGTTG CAGCACTGGC CAAAGCAGGT 1200 CTTTTCTACA CAGGTATAAA GGACATCGTC CAGTGCTTTT CCTGTGGAGG GTGTTTAGAG 1260 AAATGGCAGG AAGGTGATGA CCCATTAGAC GATCACACCA AGAACCTCTG GTGCTGCCTG AGGTCTTTGG CAACTTGAAC 1680 TCTGTCATGT GTGTGGAGGG TGAAGCTGGA ATCTGGCCAC GGACCACTTG CTGGGCTGTG ATCTGTCTAT TGCTTCAAAA 1620 CACATCAGCA AACCTGTGCA AGAGGAGCAG 420 AAGGAGCGAG CAAAAATGCA GAAAGGCTAC AACTCTCAAA TGCGCAGTGA AGCAAAAAGG AGTGGAAAGA CGGTCCTCCT GAAGAAATA 1740 GCTTTTCTGT GGGCATCTGG ATGCTGTCCC CTGTTAAACA CAGCTTATTG CAATGACAGC ATCTTTGCTT ACGAAGAACT ACGGCTGGAC 1140 TCTTTTAAGG ACTGGCCCCG AATGAGCAGC TGAGAGCAGC TTATACCAGC GCCAGTTTCC GCCACATGTC TTTGCTTGAT 1560 ATCTCTTCCG GATGITITICS CAATIGICSA 1320 TITCICSAAA ATAIGAAGIS CISTGSGGAA GIGASICSAG ASSITOAGAG ACTGTGGATA AACCTCAGAA AATGGCCACC 300 CAGCAGAAAG CCTCTGACGA GAGGATCTCC CAGTTTGATC GGATGTTGGT AACATTGCCA AGTACGACAT AAGGGTGAAG 720 AATCTGAAGA GCAGGCTGAG AGGAGGTAAA CCGTGGTGAA 1380 CTTTGTGAAT TACTGGAAAC CACAAGTGAA AGCAATCTTG AAGATTCAAT AGCAGTTGGT AAGGGATTTG TTGACATAAC GGGAGAACAT TTTGTGAATT CCTGGGTCCA GAGAGAATTA 1080 CCTATGGCAT ACAATTTGCT GCCAGAGCTG 360 TCTGCTCTTC TGGGCCTAGA TGCAGTTCAG TTGGCAAAGG AACTAGAAGA CCGGCCTCAC GAGACTCCCC ATAGAAGACC ACAAGAGGTT TCATCCAGAT 660 TGTGGGTTCC TTTTGAACAA TGGTGGATGT 900 TTAGGAAATT GGGAAGAAGG AGATGATCCT TGGAAGGAAC ATGCCAAATG GTTCCCCAAA IGCCTGTTCA TCTACGACGA ACCCCGGGTA TTGACCCCAG ACAACAATGC CACTTCATAT 120 TGGGGACTTC CCCCTTGTGT GCTCTCAGAG 840 GCTGGCTTTG TCTTTACAGG TAAACAGGAC ACGGTACAGT GTTTTTCCTG ATGAGGTACC AAGAAGAGGA GGCTAGACTT 780 GCATCCTTCA GGAACTGGCC ATTTTATGTC CAAGGGATAT double strand topology: -- class [of straight chain-like array]: -- cDNA to mRNA origin living thing name: -- Homo GCTGGGTTTT ACTTCACTGG GGTAAAATCT GGGATTCAGT GCTTCTGCTG TAGCCTAATC 600 CTCTTTGGTG GGACAGAGCA TTTGTTCTTC AGCCACATAC TTTCCTTCCA 240 CTGGCCAGCA TTCTCCTCTA TTAGACTAGA GTCTGGGATT CCAAGGTGCA TTCATTGCAA AGTTCCTTAA ATATTTTCTC 180 ACTGCTTCCT ACTAAAGGAC sapiens array ACAAAAGGTC CTGTGCTCAC CTGGGACCCT TCTGGACGTT GCCCTGTGTT CCTCTTCGCC 60 1440 CCTATAGTGC CAGAAATGGC ACAGGGTGAA GCCCAGTGGT TTCAAGAGGC AAAGAATCTG 1500 960 TGTGAATTTC TTCGGAGTAA GAAATCCTCA GAGGAAATTA CCCAGTATAT TCAAAGCTAC 1020 480 TTAAAGACTT TTGTGACTTA TGAGCCGTAC AGCTCATGGA TACCACAGGA GATGGCGGCC 540

GAACTCAGCG CAGCCGAACA GGAACTGCTT CTCACCCTGC CTTCCCTGGA ATCTCTTGAA 3540 GTCTCAGGGA SGTTCCAGCT GGTTTTCTAC 1800 CTCTCCCTTA GTTCCACCAG ACCAGACGAG GGGCTGGCCA GTATCATCTG AGTCCTGCCT TCCAAGAATT TCTTGCGGGG ATGAGGCTGA TTGAACTCCT GGATTCAGAT 2580 AGGCAGGAAC GGAACAAAGC GACAGCTGAA 2340 ATTCTCAAAG CAACTGTGTC CTCCTGTGGT GAGCTGGCCT TGAAAGGGTT ITCGACCACC CAGAAAGCTT GTCATTGTTG AGGAGCATCC ACTTCCCAAT ACGAGGAAAT 3060 AAGACATCAC AATGACAGTT 3360 TTCTCAGCTT CACAGCGCAT CGAACTCCAT TTAAACCACA GCAGAGGCTT TATAGAAAGC AACAGGGCCA GGGACATCCG CCGATACCTA GAGACCATTC TAGAGATCAA AGCATTTCCC 2100 TTTTATAATA ATCAAGATTT GGGACTGTAT CATTTGAAAC AAATCAACTC ACCCATGATG 2640 ACTGTAAGCG CCTACAACAA CCAGAGCACA TTTTCAGTT CTGGAAACAT GTTTTGACAA ATCACAGGTG 3120 CCAACTATAG ATCAGGACTA IGCTTCTGCC TTTGAACCTA TGAATGAATG GGAGCGAAAT 3180 TTAGCTGAAA AAGAGGATAA TGTAAAGAGC FATATGGATA TGCAGCGCAG GGCATCACCA 3240 GACCTTAGTA CTGGCTATTG GAAACTTTCT CCAAAGCAGT CAATCCAGTC ACAAGACCAA ATCTTCCTA ATCTGGATAA GTTCCTGTGC 3600 CTGAAAGAAC TGTCTGTGGA TAGTGGATA ACAAAGAGTC ATTGGAGAAT 2760 ATATCTGAAA ATGATGACTA CTTAAAGCAC CAGCCAGAAA SCAGTTAAAG 1920 AATCAGGTCT TATTCCTTTT AGATGACTAC-AAAGAAATAT GTTCAATCCC-TCAAGTCATA AAAGAACCAA AGTTTGCAGA AGATACAGAA AACTCCTCTC 2220 TTTGTGGCGG CGATCTGTGC TCATTGGTTT ITTITCATGT 2400 TGCTTTGAGT TTAATGATGA TGATCTCGCA GAAGCAGGGG TTGATGAAGA TGAAGATCTA ACAAGATTCC CTGTCTAGAA 3300 GTCGATGTGA ATGATATTGA TGTTGTAGGC CAGGATATGC TTGAGATTCT ITTTTGAAC TATGTCTCCA GCCTCCCTTC AACAAAGCA 2700 GGGCCCAAAA TTGTGTCTCA TTTGCTCCAT ITTCACTGCA GATGCAGTTA 2820 CTTAGGGGAT TGTGGCAAAT TTGTCCACAA GCTTACTTTT CAATGGTTTC CAGTATCCTT TTGACCCATC CTTTGATGAT 2280 GTGGCTGTTT TCAAGTCCTA TATGGAACGC CTTTCCTTAA AGAACATTTA 2880 CTGGTTCTTG CCCTGAAAAC TGCTTATCAA AGCAACACTG TTGCTGCGTG TTCTCCATTT CTGTCTGTAT ATTACGGAAG CTCTTTTCAC ATAATATGAC TCGTCTGCGA 2160 AAGTTTATGG TTTACTTTGG 980 GGAAAACTGA TTCAAAAAA CCACTTATCC-CGGACCTGCC TATTGATTGC-TGTCCGTACA 2040 3420 ATCCGCCCAG CTCTTGAGCT GTCTAAGGCC TCTGTCACCA AGTGCTCCAT AAGCAAGTTG 3480 2460 ACCATGTGCT TGATGAGCAA ATTTACAGCC CAGAGACTAA GACCATTCTA CCGGTTTTTA 2520 2940 GTTTTGCAAT TCCTTCAAGG GAGAACACTG ACTTTGGGTG CGCTTAACTT ACAGTACTT 3000 FGACCAGCT C 1860 CTAGAGAAAG AAGGATCTGT-TACTGAAATG-TGCATGAGGA-ACATTATCCA

CCTCCATTGT 4800 CCATGGTCAA CAGGGAAGGG GTTGGGGACA GGTCTGCCAA TCTATCTAAA AGCCACAATA TGGACAACAT GCCAAACTTG 4260 CAGGAGTTGG ACATCTCCAG GCATTTCACA GAGTGTATCA AAGCTCAGGC TICATGITIT CCATCTGAAG 3780 TGTAACTICT TITCGGATIT TGGGTCTCTC ATGACTATGC TTGTTTCCTG T AAAAAACAAA ACAAAAAA ACACAGTCCT 4680 GCATACTCAC CACCAAGCTC AAGAAATAAA TCATCACCAA TACCTTTGAG GTCCCTGAGT 4740 AATCCACCCC AGCTAAAGGC AAACCCTTCA ATCAAGTTTA TACAGCAAAC GCCAATATAA AGAGGAAACA GGGGTTAGGG AAAAATGACT 5100 TCATTCCAGA GGCTTCTCAG AGTTCAACAT TCAGAAAAAT TTGCCTACAT-TTTAGGTTCT CTTAGTAACC-TGGAAGAATT GATCCTTCCT 4020 ACTGGGGATG ITTCAAGACT TTGAATGATG ACAGCGTGGT GGAAATTGCC 4140 AAAGTAGCAA TCAGTGGAGG TTTCCAGAAA CTTGAGAACC TAAAGCTTTC AATCAATCAC 4200 AAGATTACAG AGGAAGGATA CAGAAATTTC TTTCAAGCAC TCAACTCCCC TCCCCTTGCC CAAGTATGAA ATATAGGGAC AGTATGTATG GTGTGGTCTC 5460 ATTTGTTTAG CATGGATGAA ACGGGTTTAA CACAGGATCC ACATGAATCT TCTGTGGGCC AAAATATGTT 4980 CCTTAATCCT CCAGAATTTC 5280 GTTCCTACCA GTTGAGTAGT TTTCTGAACG GCCAGAAGAC CATTCGAAAT TCATGATACT GAATTTATCG AGTGGCCAAA CTGATCATCC AGCAGTGTCA GCAGCTTCAT 4080 TGTCTCCGAG TCCTCTCATT CACAACAGTC 4320 AAGTCTTTGA GTCAATGTGT GTTACGACTA CCAAGGCTCA TTAGACTGAA CATGTTAAGT ICTGGAGGGC AATATAAATG TTTTTCAGT CATTCCTGAA 3660 GAATTTCCAA ACTTCCACCA TATGGAGAAA ICTAAGTACT TAACTATTCT CCAGAAATGG ATACTGCCGT TCTCTCCAAT CATTCAGAAA 4500 TAAAAGATTC AGCTAAAAAC TGCTGAATCA ATAATTTGTC TTGGGGCATA TTGAGGATGT 4560 AAAAAAAGTT GTTGATTAAT TGTAGAACCT GTCTTCTATA TTGAACTAGC TTTGGTACAG TAGAGTTAAC 5040 TTACTTTCCA TTTATCCACT ITATTGATCC AAATTTCAGC TGAGTATGAT 3720 CCTTCCAAAC TAGTAAAATT AATTCAAAAT TCTCCAAACC AAGAAACTC 3840 ACAGAAATTA AGTTTTCGGA-TTCATTTTT CAAGCCGTCC-CATTTGTTGC CAGTTTGCCA GCTAAAAACC AAATTATCCA AAATTATTT ATTAAATATT 4620 GCATACAAAA GAAAATGTGT AAGGCTTGCT ATGCTATAAT TTAGAATTTT CTTATGAATC 5160 CACTCTACTT GGGTAGAAAA TATTTTATCT CTAGTGATTG CATATTATTT CCATATCATA 5220 GTATTTCATA GTATTATATT TGATATGAGT GTCTATATCA ATGTCAGTGT 4380 TGGCTCTTGG ATGCAGATGA TATTGCATTG CTTAATGTCA TGAAAGAAAG ACATCCTCAA 4440 3900 AATTTTATTT CTCTGAAGAT-ATTAAATCTT GAAGGCCAGC-AATTTCCTGA TGAGGAAACA 3960 4860 TGGAAGAAGT ATTCAATTTA TATAATAAAT GGCTAACTTA ACGGTTGAAT CACTTTCATA 4920 5340 ACTATAAGTT GGTAAACAAC CATACTTTTA TCCTCATTTT TATTCTCACT AAGAAAAAG 5400

GCAGGCTGAG AGGAGGTAAA ATGAGGTACC AAGAAGAGGA GGCTAGACTT 780 GCATCCTTCA GGAACTGGCC TTGGCAAAGG AACTAGAAGA AGAGGAGCAG 420 AAGGAGCGAG CAAAAATGCA GAAAGGCTAC AACTCTCAAA ICAAAGCTAC 1020 AAGGGATTTG TTGACATAAC GGGAGAACAT TTTGTGAATT CCTGGGTCCA GAGAGATTA FGCGCAGTGA AGCAAAAAGG 480 TTAAAGACTT TTGTGACTTA TGAGCCGTAC AGCTCATGGA TACCACAGGA GATGGCGGCC 540 GCTGGGTTTT ACTTCACTGG GGTAAAATCT GGGATTCAGT GCTTCTGCTG TAGCCTAATC ITCTCCTCTA TTAGACTAGA ACTGTGGATA AACCTCAGAA AATGGCCACC 300 CAGCAGAAAG CCTCTGACGA GAGGATCTCC CAGTTTGATC ACAATTTGCT GCCAGAGCTG 360 TCTGCTCTTC TGGGCCTAGA TGCAGTTCAG ACGGTACAGT GTTTTCCTG TGGTGGATGT 900 TTAGGAAATT GGGAAGAAGG AGATGATCCT TGGAAGGAAC ACTECTICCT ACTAAAGGAC GGACAGAGCA TTIGTICTIC AGCCACATAC TTICCTICCA 240 CTGGCCAGCA TGTGGGTTCC TTTTGAACAA GGATGTTGGT AACATTGCCA AGTACGACAT AAGGGTGAAG 720 AATCTGAAGA TGGTAACCAT TTATAATATC AGAAAGTATA TGTACACCAA 5880 AACATGTTGA ACATCCATGT TGTACAACTG ATTITATGIC CAAGGGATAT CCCCTIGIG GCTCTCAGAG 840 GCTGGCTTIG TCTTTACAGG TAAACAGGAC AACAAAAAC AAAACCACTT ATATTGCTAG CTACATTAAG AATTTCTGAA 5820 TATGTTACTG AGCTTGCTTG ATGCCAAATG GTTCCCCAAA 960 TGTGAATTTC TTCGGAGTAA GAAATCCTCA GAGGAAATTA CCCAGTATAT AAATATAAAT AATTTTGTCA ATTATACCTA 5940 AATAAAACTG GAAAAAAAA AAAAAAAA AAAAAAAA CCTCTTCGCC 60 TGCCTGTTCA TCTACGACGA ACCCCGGGTA TTGACCCCAG ACAACAATGC CACTTCATAT AAAA 5984 array number: -- die-length [of two arrays]: -- mold [of 5366 arrays]: -- number [of nucleic-acid chains]: -- double strand topology: -- class [of straight chain-like array]: -- cDNA to mRNA origin living thing name: -- Homo sapiens array ACAAAAGGTC CTGTGCTCAC CTGGGACCCT TCTGGACGTT GCCCTGTGTT 1080 CCTATGGCAT CAGCTTATTG CAATGACAGC ATCTTTGCTT ACGAAGAACT ACGGCTGGAC 1140 600 CTCTTTGGTG CCGGCCTCAC GAGACTCCCC ATAGAAGACC ACAAGAGGTT TCATCCAGAT 660 GCCACTGGGG CGGCTGAGAC-GCAGGACTTG CTTGAACCCG-GGAGGCAGAG 5700 GTTGCAGTGA 120 TGGGGACTTC GTCTGGGATT CCAAGGTGCA TTCATTGCAA AGTTCCTTAA ATATTTTCTC 180 GAGGCGGCC AATCATTTGA GGTGAGGAGT TCGAGACCGG CCTGGCCAGC 5 580 ATGGTGAAAC GCCGAGATGG CGCCACTGCA-TTCCAGCCTG GGCAACAGAG-CAAGACCCTG 5760 TCTGTTTCAA CCCATTTTTG CTAAAGGTAC-AAAAATTAGC CAGGTGTGGT-GGCACATGCC 5640 TGTGGTCCCA AAAACCACTT ATGACTGGGT GCGGTGGCTC ACACCTGTAA TCCCAGCACT 5520 TTGGGAGGCT

CCACTTATCC CGGACCTGCC TATTGATTGC TGTCCGTACA 2040 AACAGGGCCA GGGACATCCG CCGATACCTA FACTGGAAAC-CACAAGTGAA AGCAATCTTG-AAGATTCAAT AGCAGTTGGT 1440 CCTATAGTGC CAGAAATGGC ICTITIAAGG ACTGGCCCCG GGAATCAGCT GTGGGAGTTG CAGCACTGGC CAAAGCAGGT 1200 CTTTTCTACA AGGTCTTTGG CAACTTGAAC 1680 TCTGTCATGT GTGTGGAGGG TGAAGCTGGA AGTGGAAAGA CGGTCCTCCT CTGGGCTGTG ATCTGTCTAT TGCTTCAAAA 1620 OACATCAGCA AACCTGTGCA AGAACCTCTG GTGCTGCCTG ITAATGATGA TGATCTCGCA GAAGCAGGGG TTGATGAAGA TGAAGATCTA 2460 ACCATGTGCT TGATGAGCAA TTATACCAGO GCCAGTTTCC GCCACATGTC TTTGCTTGAT 1560 ATCTCTTCCG ATCTGGCCAC GGACCACTTG GAAGAAAATA 1740 GCTTTTCTGT GGGCATCTGG ATGCTGTCCC CTGTTAAACA GGTTCCAGCT GGTTTCTAC ATTTACAGCC CAGAGACTAA GACCATTCTA CCGGTTTTTA 2520 AGTCCTGCCT TCCAAGAATT TCTTGCGGGG ATGAGGCTGA TTGAACTCCT GGATTCAGAT 2580 AGGCAGGAAC ATCAAGATTT GGGACTGTAT CATTTGAAAC AACAAAAGCA 2700 GGGCCCAAAA TTGTGTCTCA TTTGCTCCAT TTAGTGGATA ACAAAGAGTC ATTGGAGAT AAATCAACTC ACCCATGATG 2640 ACTGTAAGCG CCTACAACAA TTTTTGAAC TATGTCTCCA GCCTCCCTTC CTAGAGAAAG AAGGATCTGT TACTGAAATG TGCATGAGGA ACATTATCCA GCAGTTAAAG 1920 AATCAGGTCT CCCTGAAAAC TGCTTATCAA AGCAACACTG TTGCTGCGTG TTCTCCATTT 2940 GTTTTGCAAT TCCTTCAAGG ATAATATGAC TCGTCTGCGA 2160 AAGTTTATGG TTTACTTTGG AAAGAACCAA AGTTTGCAGA AGATACAGAA ATTCTCAAAG CAACTGTGTC CTCCTGTGGT GAGCTGGCCT TGAAAGGGTT TTTTCATGT 2400 TGCTTTGAGT TATTCCTTTT AGATGACTAC AAAGAAATAT GTTCAATCCC TCAAGTCATA 1980 GGAAAACTGA TTCAAAAAA GAGACCATTC TAGAGATCAA AGCATTTCCC 2100 TTTTATAATA CTGTCTGTAT ATTACGGAAG CTCTTTTCAC AACTCCTCTC 2220 TTTGTGGCGG CGATCTGTGC TCATTGGTTT CAGTATCCTT TTGACCCATC CTTTGATGAT CTTAGGGGAT TGTGGCAAAT TTGTCCACAA GCTTACTTTT CAATGGTTTC AGAACATTTA 2880 CTGGTTCTTG 1800 CTCTCCCTTA GTTCCACCAG ACCAGACGAG GGGCTGGCCA GTATCATCTG TGACCAGCTC 1860 2280 GTGGCTGTTT TCAAGTCCTA TATGGAACGC CTTTCCTTAA GGAACAAAGC GACAGCTGAA 2340 2760 ATATCTGAAA ATGATGACTA CTTAAAGCAC CAGCCAGAAA TTTCACTGCA GATGCAGTTA 2820 ATATGAAGTC-CTCTGCGGAA GTGACTCCAG-ACCTTCAGAG CCGTGGTGAA 1380 CTTTGTGAAT CAGGTATAAA GGACATCGTC CAGTGCTTTT CCTGTGGAGG GTG TTTAGAG 1260 AAATGGCAGG ACAGGGTGAA GCCCAGTGGT TTCAAGAGGC AAAGAATCTG 1500 AATGAGCAGC TGAGAGCAGC AAGGTGATGA-CCCATTAGAC GATCACACCA-GATGTTTTCC CAATTGTCCA 1320 TTTCTCCAAA

ATCCGCCCAG CTCTTGAGCT GTCTAAGGCC TCTGTCACCA AGTGCTCCAT AAGCAAGTTG 3480 GAACTCAGCG CAGCCGAACA GGAACTGCTT CTCACCCTGC CTTCCCTGGA ATCTCTTGAA 3540 GTCTCAGGGA CAATCCAGTC AAACTCTGCC TCCTGGGTTC AAGCGATTCT CCTGCCTCAG 4620 CCTCCCAAGT AGCTAGGATT ACAGGTGAAC TTGAATGATG ACAGCGTGGT GGAAATTGGT 4140 GAGCTAGTGT TTCAGCTTGC ATGGAAGCCA GTGGTATAGC TGCAGCGCAG GGCATCACCA 3240 GACCTTAGTA CTGGCTATTG GAAACTTTCT CCAAAGCAGT ACAAGATTCC ITTGAACCTA-TGAATGAATG GGAGCGAAAT 3180 TTAGCTGAAA AAGAGGATAA-TGTAAAGAGC TATATGGATA SAGAACACTG ACTTTGGGTG CGCTTAACTT ACAGTACTTT 3000 TTCGACCACC CAGAAAGCTT-GTCATTGTTG ACAAGACCAA ATCTTCCTA ATCTGGATAA GTTCCTGTGC 3600 CTGAAAGAAC TGTCTGTGGA TCTGGAGGGC GCCACCACAC CTGGCTAATT TTGTATTTTT 4680 AGTAAACACA GGGTTTCACC ATGTTGGCCA GGCTAGTCTC AGTGGCCAAA CTGATCATCC AGCAGTGTCA GCAGCTTCAT 4080 TGTCTCCGAG TCCTCTCATT TTTCAAGACT GTTTTAGACA CCTGGCCACA TACTCTCCTA AGTACTCCTT TTTAAAACTG AAGATGAATA 4440 TACACACAGA CTGTCTAGAA 3300 GTCGATGTGA ATGATATTGA TGTTGTAGGC CAGGATATGC TTGAGATTCT AATGACAGTT ITGCCTACAT TTTAGGTTCT CTTAGTAACC TGGAAGAATT GATCCTTCCT 4020 ACTGGGGATG GAATTTATCG ITCACTATCA 4260 TACTGTTCCT TCTAGTGTCC TTCTGTGGAT TTAGGCGCAT TCTGGTCAGA TTTGGAAGTA CAAGCTTTCT GCTGCAACAT 4200 GTCTATGTAA ACATTTGCCC CTCTAGAAAT TTTCAACCCG CTTCCTCATT CCATCTGAAG 3780 TGTAACTTCT TTTCGGATTT TGGGTCTCTC ATGACTATGC TTGTTTCCTG TAAGAAACTC AATTTTATTT CTCTGAAGAT ATTAAATCTT GAAGGCCAGC AATTTCCTGA TGAGGAAACA 3960 TCAGAAAAAT AATATAAATG TTTTTCAGT CATTCCTGAA 3660 GAATTTCCAA ACTTCCACCA TATGGAGAAA TTATTGATCC AAATTTCAGC TGAGTATGAT 3720 CCTTCCAAAC TAGTAAAATT AATTCAAAAT TCTCCAAACC TTCATGTTTT AAAGTACAAA AATCATGTG ACTGCTCACT GAATTTTATT TTCTTATTTT 4500 CTTCTTTTT TTTTTTGA 3360 TTCTCAGCTT CACAGCGCAT CGAACTCCAT TTAAACCACA GCAGAGGCTT TATAGAAAGC 3420 4320 CAAAAAGGTC TCCCATTTGT GGATATACAA GCCCTCAAAT CTGCGTTCTT GCCACCTGGT 4380 3840 ACAGAAATTA AGTTTTCGGA TTCATTTTT CAAGCCGTCC CATTTGTTGC CAGTTTGCCA 3900 GACAGAGTTT CGCTCGTGTT GCCCAGGCTG GAGTACAATG 4560 GCACGATCTC GGGTCACTGC GAACTCCTGA CCTCAAGTGA 4740 GCCACAGTGC CTGGCCTGAG GAACTGAGAT TTCTGTCGAG CTGGAAACAT-GTTTTGACAA ATCACAGGTG 3120 CCAACTATAG ATCAGGACTA-TGCTTCTGCC AGGAGCATCC-ACTTCCCAAT ACGAGGAAAT 3060 AAGACATCAC CCAGAGCACA-TTTTCAGTI

ACCTGAAGGG AGAATGGCCC 4800 AGGCATAGTT GGTAGAGGAG GAATTGAGAC ATCATTTCAA ACAGAGGTAA CATCCTGGCT 5160 AACATGGTGA AACCCCGTCT CTACTAAAAA TACAAAAAT TAGCCAGGCG TGATGGCGGG 80 Met Ala Ala Ala Gly Phe Tyr Phe Thr Gly Val Lys Ser Gly Ile Gln 85 9095 Cys Phe Cys CysSer Leu Ile LeuPhe Gly Cys Gly Gly Cys Leu Gly Asn Trp Glu 195 200 205 Glu Gly AspAsp Pro Trp Lys Glu His Ala Lys Trp Phe Pro Lys Cys Ser Asp Glu Arg Ile Ser Gln Phe Asp 1 5 10 15 His Asn Leu Leu Pro Glu Leu Ser Ala Leu Leu Gly Leu Asp Ala Val 20 arrays]: -- amino acid topology: -- class [of straight chain-like array]: -- protein array Met Ala Thr Gln Gln Lys Ala Met Arg Ser Glu Ala Lys Arg Leu 50 55 60 Lys Thr Phe ValThr Tyr Glu Pro Tyr Ser Ser Trp Ile Pro Gln Glu 65 70 75 Ala Gly Leu Thr Arg Leu 100 105 110 Pro Ile Glu Asp His Lys ArgPhe His Pro Asp Cys Gly Phe Leu Leu 115 120 125 170 175 Ser Pro Cys Val Leu Ser Glu Ala Gly Phe Val Phe Thr Gly Lys Gln 180 185 190 Asp Thr ValGln Cys Phe Ser Met Arg Tyr Gln Glu Glu Glu 145 150 155 160 Ala Arg Leu Ala Ser Phe Arg Asn Trp Pro Phe Tyr Val Gln Gly Ile 165 GluSerAla Val Gly Val Ala Ala Leu Ala Lys Ala Gly Leu 290 295 300 Phe Tyr Thr Gly Ile Lys Asp Ile Val Gln Cys Phe ICTCATTITC-TTACTGCTCA 5040 GCACTGTTAT TTTACGTTAT TTAAAACAGC TGGGAGCGGT GGCTCAAGCT Arg Gly Glu Leu Cys Glu Leu Leu 355 360 365 Glu Thr ThrSer Glu Ser Asn Leu Glu Asp Ser Ile Ala Val Gly Pro 370 Asn Lys Asp Val Gly AsnIle Ala Lys Tyr Asp Ile Arg Val Lys Asn 130 135 140 Leu Lys Ser Arg Leu Arg Gly Gly Lys 210 215 220 Glu Phe Leu Arg Ser Lys Lys Ser Ser Glu Glu Ile Thr Gln Tyr Ile 225 230 235 240 Gln Ser Tyr Lys Gly 25 30 Gln Leu Ala Lys Glu Leu Glu Glu Glu Glu Glu Glu Hys Glu Arg Ala Lys 35 40 45 Met Gln Lys Gly Tyr Asn Ser Gln Phe Val Asp Ile-Thr-Gly-Glu-His Phe Val Asn 245 250 255 Ser Trp Val Gln Arg Glu Leu Pro Met-Ala-Ser-Ala-Tyr Ser Cys Gly Gly 305 310 315 320 Cys Leu Glu Lys Trp Gln Glu Gly Asp Asp Pro Leu Asp Asp His Thr 325 330335 Arg Cys PheProAsn Cys Pro Phe Leu Gln Asn Met Lys Ser Ser Ala 340 345 350 Glu Val Thr Pro Asp Leu Gln Ser Cys Asn Asp 260 265 270 Ser Ile Phe Ala Tyr Glu Glu Leu Arg Leu Asp Ser Phe Lys Asp Trp275 280285 Pro Arg TCCGTCTCAA AAAAAAAA CAAAAA 5366 array number: -- die-length [of three arrays]: -- mold [of 1404 5220 CACCTGTAGT CCCAGCTACT CGGGAGGCTG AGGCAGGAGA ATGGTGTGAA CCCGGGGAGGT 5280 CTTGGGGGAA-AAAAAGGAAT 4920 GTCTGGAGCA AGAGGCAGGA GTGAGTTGTG-AGAAGAGAC FGGAGAGGAA-AGTAAAAGCC 4980 CAATTGGAGA GGCTTTGTCG GGTGTGTTAC-AAGGGCTGGA IGTAATCCCA 5100 GCACTTTGGG AGGCCGAGGC GGATGGATCA CGAGGTCAGG AGATCGAGAC GGAGCTTGAA GTGAGCCAAG ATCATGCCAC TGCACTCCAG CCTGGGCAAC AGAACGAGAC 5340 ICACTTGTGT 4860 CATAGCCTGG-AGTTAAAGAG AACCAGATAT-ATTTGAAGAA

Lys Gln Ile Asn Ser Pro Met Met Thr 770 775 780 Val Ser Ala Tyr Asn Asn Phe Leu Asn Tyr Val Ser Ser Leu Pro Ser His 420 425 430 Leu Leu GlyCysAsp Leu Ser Ile Ala Ser Lys His Ile Ser Lys Pro 435 440 445 Val Gln Glu Pro Leu Val Gln-Gly-Arg-Thr-Leu Thr Leu Gly 885 890 895 Ala Leu Asn Leu Gln Tyr Phe Phe Asp-His-Pro-Glu Ser Leu Ser Leu Glu Thr Cys Phe Asp Lys Ser Gln Val Pro 930 935 940 Thr Ile Asp Gln Asp Tyr Ala Ser Ala Phe Glu Pro Met Asn Glu Leu Pro Glu Val Phe Gly Asn Leu Asn Ser 450 455 460 Val Met Cys Val Glu Gly Glu Ala Gly Ser Gly Lys Thr Val Leu Lys 625 630 635 640 Thr Pro Leu Phe Val Ala Ala Ile Cys Ala His Trp Phe Gln Tyr Pro 645 650 655 Phe Asp Pro Ser 745 750 Gly Met ArgLeu Ile Glu Leu Leu Asp Ser Asp Arg Gln Glu His Gln 755 760 765 Asp Leu Gly Leu Tyr His Leu Phe Ser His Asn Met Thr Arg Leu Arg Lys 610 615 620 Phe Met ValTyr Phe Gly LysAsn Gln Ser Leu Gln Lys Ile Gln 785 790 795 800 Thr Lys Ala Gly Pro Lys Ile Val Ser His Leu Leu His Leu Val Asp 805 810 815 Asn Lys Glu Ser Leu Leu Arg Ala Ala Tyr Thr Ser Ala Ser Phe 405 410 415 Arg His Met Ser LeuLeu Asp Ile Ser Ser Asp Leu Ala Thr Asp Glu Asn Ile Ser Glu Asn Asp Asp Tyr Leu Lys 820 825 830 His Gln Pro Glu Ile Ser Leu Gln Met Gln Leu Leu Arg Gly ²he Asn Asp Asp Leu Ala Glu Ala Gly Val Asp Glu Asp 705 710 715 720 Glu Asp Leu Thr Met Cys Leu Met Ser Phe Asp Asp Val Ala Val Phe Lys Ser Tyr Met Glu 660 665 670 Arg Leu SerLeuArg Asn Lys Ala Thr Ala Glu Ile Leu Arg Ala Ser Pro Asp Leu Ser Thr Gly Tyr Trp Lys Leu 980 985 990 Ser Pro LysGln Tyr Lys Ile Pro Cys Leu Glu Val 375 380 Ile Val Pro Glu Met Ala Gln Gly Glu Ala Gln Trp Phe Gln Glu Ala 385 390 395 400 Lys Asn Leu Asn Glu Gln Glu Lys Glu Gly 515 520 525 Ser Val Thr Glu Met Cys Met Arg Asn Ile Ile Gln Gln Leu Lys Asn 530 535 540 Gln Val Leu Val Phe Tyr Leu Ser Leu Ser Ser Thr Arg Pro Asp 500 505 510 Glu Gly LeuAla SerIle Ile Cys Asp Gln Leu Leu Lys-Asn-His-Leu-Ser Arg Thr Cys 565 570 575 Leu Leu Ile Ala Val Arg Thr Asn Arg-Ala-Arg-Asp-Ile Arg Arg Tyr Leu Trp 835 840 845 Gln Ile CysProGln Ala Tyr Phe Ser Met Val Ser Glu His Leu Leu 850 855 860 Val Leu Ala Leu 900905 910 Leu Arg Ser Ile His Phe Pro Ile Arg Gly Asn Lys Thr Ser Pro Arg 915 920 925 Ala His Phe Ser Val Leu Trp 945 950 955 960 Glu Arg Asn Leu Ala Glu Lys Glu Asp Asn Val Lys Ser Tyr Met Asp 965 970 975 Met Gln Arg Lys Ala Thr 675 680 685 Val Ser Ser Cys Gly Glu Leu Ala Leu Lys Gly Phe Phe Ser Cys Cys 690 695 700 Phe Glu Lys Phe Thr Ala Gln Arg Leu 725 730 735 Arg Pro Phe Tyr Arg Phe Leu Ser Pro Ala Phe Gln Glu Phe Leu Ala 740 Leu 465 470 475 480 Lys Lys Ile Ala Phe Leu Trp Ala Ser Gly Cys Cys Pro Leu Leu Asn 485 490 495 Arg Phe Gln 580 585590 Leu Glu ThrIle Leu Glu Ile Lys Ala Phe Pro Phe Tyr Asn Thr Val 595 600 605 Cys Ile Leu Arg Lys Leu Leu Phe Leu Leu Asp Asp Tyr Lys Glu Ile Cys Ser Ile Pro 545 55 0 555 560 Gln Val Ile Gly Lys Leu Ile Gln Lys Thr Ala Tyr Gln Ser Asn Thr Val Ala Ala Cys 865 870 875 880 Ser Pro Phe Val Leu Gln Phe Leu

Ala Lys Arg Leu 50 55 60 Lys Thr Phe Val Thr Tyr Glu Pro Tyr Ser Ser Trp-Ile-Pro-Gln-Glu 65 70 75 80 Met Ala Ala Ala-Gly-Phe-Tyr-Phe-Thr Gly Val Lys Ser Gly-Ile-Gln 85 90 95 Cys Phe Cys Cys-Ser-Leu-Ile-Leu-Phe Gly Ala Gly Gin Ile Phe Pro Asn Leu Asp Lys Phe Leu Cys Leu 1090 1095 1100 Lys Giu Leu Ser Val Asp Leu Glu Gly Asn Ile Asn Ser Ala Ser Gln Arg IleGlu Leu His Leu Asn His Ser Arg Gly Phe 1025 1030 1035 1040 Ile Glu Ser Ile Arg Pro Ala Leu Prolle Ile Gln Lys 1395 1400 1403 array number: -- die-length [of four arrays]: -- mold [of 1295 arrays]: -- amino Ala Lys Glu Leu Glu Glu Glu Glu Glu Gln Lys Glu Arg Ala Lys 35 40 45 Met Gln LysGly Tyr Asn Ser Gln Met Arg Ser Glu acid topology: -- class [of straight chain-like array]: -- protein array Met Ala Thr Gln Gln Lys Ala Ser Asp Glu Arg Gly Phe Gln Lys Leu Glu Asn 1285 1290 1295 Leu Lys Leu Ser Ile Asn His LysIle Thr Glu Glu Gly Tyr Arg Asn 1300 Arg Tyr Gln Glu Glu Glu 145 150 155 160 Ala Arg Leu Ala Ser Phe Arg Asn Trp Pro Phe Tyr Val Gln Gly Ile 165 170 Asp Gly Ile 1235 12401245 Tyr Arg ValAla Lys Leu Ile Ile Gln Gln Cys Gln Gln Leu His Cys 1250 1255 1260 Leu Arg 1305 1310 Phe Phe Gln Ala Leu Asp Asn Met Pro Asn Leu Gln Glu Leu Asp Ile 1315 1320 1325 Ser Arg HisPhe Thr Lys Asp Val Gly Asn Ile Ala Lys Tyr Asp Ile Arg Val Lys Asn 130 135 140 Leu Lys Ser Arg Leu Arg Gly Gly Lys Met lle Ser Gln Phe Asp 1 5 10 15 His Asn Leu Leu Pro Glu Leu Ser Ala Leu Leu Gly Leu Asp Ala Val 20 25 30 Gln Leu Val Leu Ser Phe Phe LysThr Leu Asn Asp Asp Ser Val Val 1265 1270 1275 1280 Glu Ile Ala Lys Val Ala Ile Ser Gly 1060 1065 1070 Leu Leu ThrLeu Pro Ser Leu Glu Ser Leu Glu Val Ser Gly Thr Ile 1075 1080 1085 Gln Ser Gln Asp 1195 1200 Ser Leu Pro Asn Phe Ile Ser Leu Lys Ile Leu Asn Leu Glu Gly Gln 1205 1210 1215 Gln Phe Pro Asp Glu Glu Thr Ser Glu Lys Phe Ala Tyr Ile Leu Gly 1220 1225 1230 Ser Leu Ser Asn Leu Glu Glu Leu Ile Leu Pro Thr Gly Glu Leu Ser Lys Ala Ser Val Thr 1045 1050 1055 Lys Cys Ser Ile Ser Lys Leu Glu Leu Ser Ala Ala Glu Glu Glu Leu Glu Cys Ile Lys Ala Gln Ala Thr Thr Val Lys 1330 1335 1340 Ser Leu Ser Gln Cys Val Leu Arg Leu Pro Arg Leu Ile Leu Thr Arg Leu 100 105 110 Pro Ile Glu Asp His Lys Arg Phe His Pro Asp Cys Gly Phe Leu Leu 115 120 125 Asn Asp Val Asn Asp 995 1000 1005 Ile Asp Val Val Gly Gln Asp Met Leu Glu Ile Leu Met Thr Val Phe 1010 1015 1020 Val Phe Ser Val 1105 1110 1115 1120 Ile Pro Glu Glu Bhe ProAsn Phe His His Met Glu Lys Leu Leu Ile 1125 1130 Lys-Lys-Leu-Thr 1170 1175 1180 Glu Ile Lys Phe-Ser-Asp-Ser-Phe Phe Gln Ala Val Pro Phe Val Ala 1185 1190 Arg Leu Asn 1345 1350 1355 1360 Met Leu Ser Trp Leu Leu Asp Ala Asp Asp Ile Ala Leu Leu Asn Val 1365 1370 ValPhe His Leu Lys Cys Asn Phe Phe Ser 1155 1160 1165 Asp Phe Gly Ser-Leu-Met-Thr-Met Leu Val Ser Cys 1375 Met Lys Glu Arg His Pro Gln Ser Lys Tyr Leu Thr Ile Leu Gln Lys 1380 1385 1390 Trp Ile Leu Pro Phe Ser 1135 Gln Ile Ser Ala Glu Tyr Asp Pro Ser Lys Leu Val Lys Leu Ile Gln 1140 11451150 Asn Ser Pro Asn Leu His

Cys Phe Pro Asn Cys Pro Phe Leu Gln Asn Met Lys Ser Ser Ala 340 345 350 Glu Val ThrProAsp Leu Gln Ser Arg Gly Leu Lys Gln Ile Asn Ser Pro Met Met Thr 770 775 780 Val Ser Ala Tyr Asn Asn Phe Leu Asn Tyr Val Ser Ser Leu Pro 635 640 Thr Pro Leu Phe Val Ala Ala Ile Cys Ala His Trp Phe Gln Tyr Pro 645 650 655 Phe Asp Pro Ser Phe Asp Asp Asn Met Thr Arg Leu Arg Lys 610 615 620 Phe Met Val Tyr Phe Gly Lys Asn GlnSer Leu Gln Lys Ile Gln Lys 625 630 Cys Asn Asp 260 265 270 Ser IlePheAla Tyr Glu Glu Leu Arg Leu Asp Ser Phe Lys Asp Trp 275 280 285 Pro Arg Glu Glu Leu Cys Glu Leu Leu 355 360 365 Glu Thr Thr Ser Glu Ser Asn Leu Glu Asp Ser Ile Ala Val Gly Pro 370 375 380 eu Pro Glu Val Phe Gly Asn Leu Asn Ser 450 455 460 Val Met Cys Val Glu Gly Glu Ala Gly Ser Gly Lys Thr Val Leu Ser-Lys-Phe-Thr-Ala Gln Arg Leu 725 730 735 Arg Pro Phe Tyr-Arg Phe Leu Ser Pro Ala Phe Gln Glu Phe Leu Ala 740745 750 Gly Met Arg Leu Ile Glu Leu Leu Asp Ser Asp Arg Gln Glu His Gln 755 760 765 Asp Leu Gly Leu Tyr His Ser Ala Val Gly Val Ala Ala Leu Ala Lys Ala Gly Leu 290 295 300 Phe Tyr Thr GlylleLys Asp Ile Val Gln Cys Phe Ser 675 680 685 Val Ser Ser Cys Gly Glu Leu Ala Leu Lys Gly Phe Phe Ser Cys Cys 690 695 700 Phe Glu Phe Asn Asp Cys 210 215 220 Glu Phe Leu Arg Ser Lys Lys Ser Ser Glu Glu Ile Thr Gln Tyr Ile 225 230 235 240 Gln Ser Tyr Lys lle Val Pro Glu Met Ala Gln Gly Glu Ala Gln Trp Phe Gln Glu Ala 385 390 395 400 Lys Asn Leu Asn Glu Gln Leu Arg His Leu Ser Arg Thr Cys 565 570 575 Leu Leu Ile Ala Val Arg Thr Asn Arg Ala Arg Asp Ile Arg Arg Tyr 580 585 590 Gly Phe Val Asp Ile Thr Gly Glu His Phe Val Asn 245 250 255 Ser Trp Val Gln Arg Glu Leu Pro Met Ala Ser Ala Tyr Glu Lys Glu Gly 515 520 525 Ser Val Thr Glu Met Cys Met Arg Asn Ile Ile Gln Gln Leu Lys Asn 530 535 540 Gln Val Val Ala Val Phe Lys Ser Tyr Met Glu 660 665 670 Arg Leu Ser LeuArg Asn Lys Ala Thr Ala Glu Ile Leu Lys Ala Thr Cys Gly Gly 305 310 315 320 Cys Leu Glu Lys Trp Gln Glu Gly Asp Asp Pro Leu Asp Asp His Thr 325 330 335 Arg Leu Phe Leu Leu Asp Asp Tyr Lys Glu Ile Cys Ser Ile Pro 545 550 555 560 Gln Val Ile Gly Lys Leu Ile Gln Lys Asn Leu Glu Thr Ile LeuGlu Ile Lys Ala Phe Pro Phe Tyr Asn Thr Val 595 600 605 Cys Ile Leu Arg Lys Leu Phe Ser His 420425 430 Leu Leu Gly Cys Asp Leu Ser Ile Ala Ser Lys His Ile Ser Lys Pro 435 440 445 Val Gln Glu Pro Leu Val Leu 465 470 475 480 Lys Lys Ile Ala Phe Leu Trp Ala Ser Gly Cys Cys Pro Leu Leu Asn 485 490 495 Arg Phe Gln Leu Val Phe Tyr LeuSer Leu Ser Ser Thr Arg Pro Asp 500 505 510 Glu Gly LeuAlaSer Ile Ile Cys Asp Gln Leu Leu Cys Gly Gly Cys Leu Gly Asn Trp Glu 195 200 205 Glu Gly Asp Asp Pro Trp Lys Glu His Ala Lys Trp Phe Pro Lys 175 Ser Pro Cys Val Leu Ser Glu Ala Gly Phe Val Phe Thr Gly Lys Gln 180 185 190 Asp Thr Val Gln Cys Phe Ser Ala-Ala-Tyr-Thr-Ser Ala Ser Phe 405 410 415 Arg His Met Ser Leu Leu Asp Ile Ser Ser Asp Leu Ala ThrAspHis Asp Asp Leu-Ala-Glu-Ala-Gly Val Asp Glu Asp 705 710 715 720 Glu Asp Leu Thr Met Cys Leu Met

Ala Tyr Ile Leu Gly 1220 1225 1230 Ser Leu Ser Asn Leu Glu Glu Leu Ile Leu Pro Thr Gly Asp Gly Ile 1235 1240 1245 Gln Ile Ser Ala Glu Tyr Asp ProSer Lys Leu Val Lys Leu Ile Gln 1140 1145 1150 Asn Ser Pro Asn LeuHis Val Phe His Asn Asp 995 1000 1005 Ile Asp Val Val-Gly-Gln-Asp-Met Leu Glu Ile Leu Met-Thr-Val-Phe 1010 1015 1020 Ser Ala Leu Lys Thr AlaTyr Gln Ser Asn Thr Val Ala Ala Cys 865 870 875 880 Ser Pro Phe Val Leu Gln Phe Leu Gln Gly Arg Phe Asp Lys Ser Gln Val Pro 930 935 940 Thr Ile Asp Gln Asp Tyr Ala SerAla Phe Glu Pro Met Asn Glu Trp 945 950 1170 1175 1180 Glu IleLys Phe Ser Asp Ser Phe Gln Ala Val Pro Phe Val Ala 1185 1190 1195 1200 Ser Leu Pro 1065 1070 Leu Leu Thr Leu Pro Ser Leu Glu Ser Leu Glu Val Ser Gly Thr Ile 1075 1080 1085 Gln Ser GlnAsp Gln Ile Phe Ser Val 1105 1110 1115 1120 Ile Pro Glu Glu Phe Pro Asn Phe His His Met Glu Lys Leu Leu Ile 1125 1130 1135 Asn Phe Ile Ser Leu Lys Ile Leu Asn Leu Glu Gly Gln 1205 1210 1215 Gln Phe Pro Asp Glu Glu Thr Ser Glu Lys Phe Gly Leu Trp 835 840 845 Gln Ile Cys Pro Gln Ala Tyr Phe Ser Met Val Ser Glu His Leu Leu 850 855 860 Val Leu Ala Leu Ser Lys Ala Ser Val Thr 1045 1050 1055 Lys Cys Ser Ile Ser Lys Leu Glu Leu Ser Ala Ala Glu Glu Leu 1060 Ser 785 790 795 800 Thr Lys Ala Gly Pro Lys Ile Val Ser His Leu Leu His Leu Val Asp 805 810 815 Asn Lys Glu Ser Leu Glu Asn Ile Ser Glu Asn Asp Asp Tyr Leu Lys 820 825 830 His Gln ProGlu Ile Ser Leu Gln Met Gln Leu Leu Arg FhrLeu Asn Asp Asp Ser Val Val 1265 1270 1275 1280 Glu Ile Gly Glu Leu Val Phe Gln Leu Ala Trp Lys Pro Val Val Thr Leu Thr Leu Gly 885 890 895 Ala Leu Asn Leu Gln Tyr Phe Phe Asp His Pro Glu Ser Leu Ser Leu 900 905 910 Fyr Arg Val Ala Lys Leu Ile Ile Gln Gln Cys Gln Gln Leu His Cys 1250 1255 1260 Leu Arg Val Leu Ser Phe Phe Lys eu Lys Cys Asn Phe Phe Ser 1155 11601165 Asp Phe GlySer Leu Met Thr Met Leu Val Ser Cys Lys Lys Leu Thr Pro Asp Leu Ser Thr Gly Tyr Trp Lys Leu 980 985 990 Ser Pro Lys Gln Tyr Lys Ile Pro Cys-Leu-Glu-Val-Asp Val Phe Pro Asn Leu Asp Lys Phe Leu Cys Leu 1090 1095 1100 Lys Glu Leu Ser Val Asp Leu Glu Gly Asn Ile Asn Val Leu ArgSerlle His Phe Pro Ile Arg Gly Asn Lys Thr Ser Pro Arg 915 920 925 Ala His Phe Ser Val Leu Glu Thr Cys Ser Gln Arg Ile Glu Leu His Leu Asn His Ser Arg Gly Phe 1025 1030 1035 1040 Ile Glu Ser Ile Arg Pro Ala Leu Glu 955 960 Glu Arg Asn Leu Ala Glu Lys Glu Asp Asn Val Lys Ser Tyr Met Asp 965 970 975 Met Gln Arg Arg Ala Ser

[Translation done.]

* NOTICES *

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2.*** shows the word which can not be translated.

3.In the drawings, any words are not translated

DESCRIPTION OF DRAWINGS

Brief Description of the Drawings

Drawing 1] It is the mimetic diagram having shown the development process of the ovarian follicle in the ovary.

Drawing 2] It is the mimetic diagram having shown the gene location of the probe for hybridization.

Drawing 3] in situ hybridization which investigated the NAIP gene expression in the ovary It is a result and is (A). It is

(B) when a sense RIBOPU lobe is used. The case where an antisense RIBOPU lobe is used is shown.

8:heart. (B) The gene expression within the ovary in the development process of a ** mouse is shown, and each lane each organization is shown, and each lane is 1:testis, 2:kidney, 3:skeletal muscle, 4:liver, 5:lungs, 6:spleen, 7:brain, and Drawing 4] It is as a result of Northern blot analysis of a mouse NAIP gene. (A) The gene expression in ** mouse is 1:2 age in day, 2:3 weeks old, 3:12 weeks old, and the 3rd day of 4:delivery and 5:18 weeks old

[Drawing 5] It is as a result of in situ hybridization which showed that the NAIP gene expression in the ovary was

carrying out localization to the granulosa cell, and (an upper case, interruption) a TUNEL assay (lower berth)

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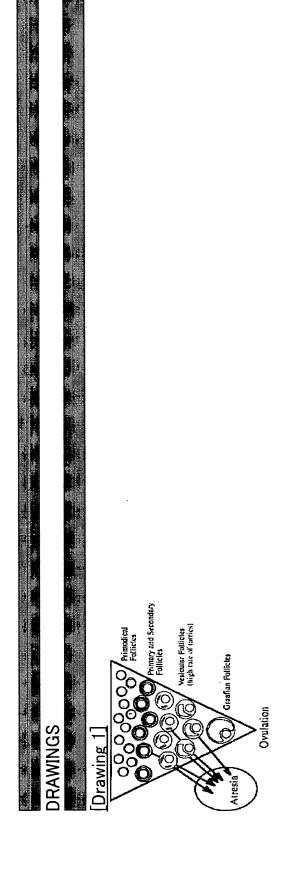
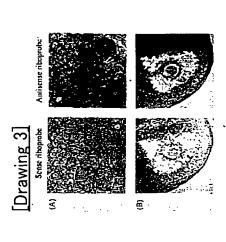
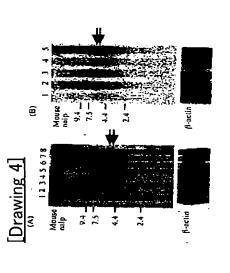


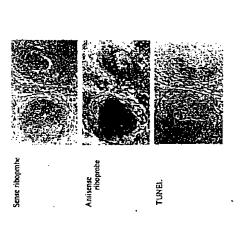
Fig. 1. Follicular development in the mouse ovary

[Drawing 2]





[Drawing 5]



[Translation done.]

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(54) 【発明の名称】 超過排卵動物と超過排卵方法

(57)【要約】

【課題】 卵胞の退行・閉鎖を抑制するNAIP遺伝子の発現を人為的に制御可能な動物個体と、NAIP遺伝子の発現を制御する方法を提供する。

【解決手段】 プロモーター配列と配列番号1または2のDNA配列とを含むDNA断片を導入した分化全能性細胞を個体へと発生させたトランスジェニック非ヒト動物およびその子孫動物であって、体細胞染色体中に上記DNA断片を保有することを特徴とする超過排卵動物と、この動物の導入遺伝子を発現させて配列番号3または4のアミノ酸配列を有するタンパク質を産生させることを特徴とする超過排卵方法。

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【特許請求の範囲】

【請求項1】 プロモーター配列と配列番号1または2.のDNA配列とを含むDNA断片を導入した分化全能性細胞を個体へと発生させたトランスジェニック非ヒト動物およびその子孫動物であって、体細胞染色体中に上記DNA断片を保有することを特徴とする超過排卵動物。

【請求項2】 プロモーター配列が、性腺刺激ホルモン のレセプター遺伝子のプロモーター配列である請求項1 の超過排卵動物。

【請求項3】 請求項1記載の超過排卵動物の排卵を人 10 為的に促進させる方法であって、プロモーター配列の転写制御因子を動物に投与し、配列番号3または4のアミノ酸配列を有するタンパク質を発現させることを特徴とする超過排卵方法。

【請求項4】 請求項2記載の超過排卵動物の排卵を人為的に促進させる方法であって、性腺刺激ホルモンを動物に投与し、配列番号3または4のアミノ酸配列を有するタンパク質を発現させることを特徴とする超過排卵方法。

【請求項5】 プロモーター配列と配列番号1または2のDNA配列とを含むDNA断片を含有する組換えベクターにより形質転換した卵母細胞を卵巣中に導入された超過排卵動物。

【請求項6】 プロモーター配列が、性腺刺激ホルモンのレセプター遺伝子のプロモーター配列である請求項5の超過排卵動物。

【請求項7】 請求項5記載の超過排卵動物の排卵を人為的に促進させる方法であって、プロモーター配列の転写制御因子を動物に投与し、配列番号3または4のアミノ酸配列を有するタンパク質を発現させることを特徴と 30 する超過排卵方法。

【請求項8】 請求項6記載の超過排卵動物の排卵を人 為的に促進させる方法であって、性腺刺激ホルモンを動 物に投与し、配列番号3または4のアミノ酸配列を有す るタンパク質を発現させることを特徴とする超過排卵方 法。

【請求項9】 ナイーブ動物個体の排卵を人為的に促進させる方法であって、タンパク質リン酸化酵素抑制因子またはスタウロスポリンもしくはそれらの誘導体を動物個体に投与することによって、配列番号3または4のアミノ酸配列を有するタンパク質を発現させることを特徴とする超過排卵方法。

【請求項10】 プロモーター配列と配列番号1のDNA配列とを含むDNA断片を含有した組換えベクター。 【請求項11】 性腺刺激ホルモンのレセプター遺伝子のプロモーター配列と配列番号1のDNA配列とを含むDNA断片を含有した組換えベクター。

【請求項12】 プロモーター配列と配列番号2のDN A配列とを含むDNA断片を含有した組換えベクター。 【請求項13】 性腺刺激ホルモンのレセプター遺伝子 50 のプロモーター配列と配列番号2のDNA配列とを含む DNA断片を含有した組換えベクター。

【請求項14】 請求項1または2記載の超過排卵動物より単離された細胞。

【請求項15】 細胞が、生殖細胞である請求項14の 細胞。

【請求項16】 プロモーター配列と配列番号1または2のDNA配列とを含むDNA断片を含有する組換えベクターにより形質転換した卵母細胞。

【請求項17】 プロモーター配列が、性腺刺激ホルモンのレセプター遺伝子のプロモーター配列である請求項16の卵母細胞。

【発明の詳細な説明】

[0001]

【発明の属する技術分野】この発明は、成熟卵子の排卵数を増加させることのできる遺伝子導入超過排卵動物と、この超過排卵動物またはナイーブ動物の排卵数を人為的に制御する方法に関するものである。

[0002]

【従来の技術】哺乳動物の雌個体の卵巣では、胎生期または生後に多くの原始卵胞(primodial follicles)が形成される。この原始卵胞は、将来卵子となる生殖細胞である卵母細胞とそれを取り巻く顆粒膜細胞で構成されるもので、これらの原始卵胞は個体の生涯を通じて性周期ごとに決まった数が一次卵胞(primary follicles)、二次卵胞(secondary follicles)、胞状卵胞(antral follicles あるいはvesicularfollicles)、そしてグラーフ卵胞(graafian follicles)へと発育していき、最後には卵母細胞が成熟して排卵(ovulation)に至る過程を踏む。しかし、出生時の原始卵胞の数は動物種によって限られており、図1に示したように、極わずかの原始卵胞が排卵過程に達するのみであり、一部は休止状態で終わるものの、99.9%の原始卵胞は、発育途上で「卵胞閉鎖」(atresia)と言われる過程を介して退行していく。

【0003】卵胞が退行する要因としては、年齢、繁殖 周期、妊娠、泌乳、卵巣内外のホルモンバランス、栄 養、局所貧血等が挙げられているものの、その詳細な分 子メカニズムは長い間不明であった。近年、卵胞閉鎖の 際に観られる形態学的、生化学的、組織学的変化の観察 から、閉鎖卵胞の顆粒膜細胞に核クロマチンの濃縮や核 の断片化が認められ、卵胞の閉鎖とアポトーシスの関係 が示唆されている。細菌の研究から、性腺刺激ホルモン がラットの閉鎖卵胞におけるアポトーシスを抑制するこ と(Tilly et al.,「文献名、雑誌名、vol. page 」 199 2; Chun et al., Endocrinology 135: 1845-1853, 1994; Tilly et al., Endocrinology 136: 1394-14023, 199 5; Tilly and Tilly, Endocrinology 136: 242-252, 19 95) 、またその抑制作用の一部は卵胞内の成長ホルモン を介していること(Tilly et al., Mol. Endocrinol. 6: 1942-1950, 1992; Chun et al., Endocrinology 135: 1 845-1853、1994; Tilly et al., Endocrinology 136: 1 394-14023、1995)が明らかになっている。さらに、アポトーシスが卵胞の選択機構の要となっている可能性の裏付けとして、性腺刺激ホルモンに依存した顆粒膜細胞内の活性酵素(Tilly and Tilly, Endocrinology 136: 242-252、1995)、p 5 3 等の癌抑制遺伝子(Tilly et al., Endocrinology 136: 1394-14023)、ced-3/インターロイキン-1転換酵素(ICE: interleukin-1 β converting enzyme) 関連遺伝子(Flaws et al., Endocrinology 136: 5042-5053)の変化が報告されている。

【0004】しかし、これらの要因が生殖細胞である卵 母細胞を含む卵巣内卵胞の生存・選択を制御する機構に おいていかなる機能を果たしているか、さらにアポトー シスを抑制する因子がそれらのどのように関係している かについては、詳細な解明はなされていなかった。とこ ろが、ごく最近、アポトーシスを抑制し、細胞の延命機 能を有するBc1-2関連遺伝子産物がラット卵巣内卵胞で 発現していることが報告されたが (Tilly et al., Endo crinology 136:232-241, 1995)、遺伝子ターゲッティン グによってBc1-2遺伝子を欠損させたマウスの観察か ら、原始卵胞の生存性にのみBcl- 2が関与しているこ とが示唆された (Ratts et al., Endocrinology 136:3 665-3668,1995)。このことから、原始卵胞から発育した 一次卵胞、二次卵胞、そして胞状卵胞における卵巣内卵 胞の退行・閉鎖の過程では、Bc1-2関連遺伝子産物とは 別のアポトーシス抑制因子が関与している可能性が想定 される。

【0005】一方、近年の分子生物学的手法の発達に伴い、その手法の一つであるポジショナルクローニングによって、家族性の遺伝病である脊椎性筋萎縮症候群(spi 30 nalmuscular atropy: SMA)の原因遺伝子として、神経細胞アポトーシス抑制タンパク質(nural apoptotic inhibitory protein:NAIP)が単離された(Royet al., Cell 80:167-178, 1995)。さらに、このNAIP遺伝子を種々の培養細胞に導入し、アポトーシスを誘起させる刺激を細胞に与えたところ、その細胞死が抑制されることが明らかになった(Liston et al., Nature 379:349-353, 1996)。これらの結果から、NAIPはBcl-2関連遺伝子産物の関与だけでは説明できないアポトーシスの制御機構において細胞延命的な機能を持つ因子 40である可能性が示唆された。

【0006】生体内において、アポトーシスといわれる生埋的細胞死は、無数の細胞から構成される生体の恒常性を保つ上で、不要な細胞を排除する機構として必須な現象である。動物個体におけるNAIPの発現は、神経細胞のアポトーシスを抑制するだけではなく、卵胞の退行・閉鎖を制御し、その動物種にプログラムされた排卵数を一定に保つようにも機能していると考えられる。従って、このNAIP遺伝子の発現を人為的にコントロールすることが可能になれば、ヒトにおける不妊療法をは50

じめとして、排卵誘発剤処理にもかかわらず排卵数が少数である有用動物(牛、馬等の家畜動物など)を効率よく生産することが可能となる。

【0007】この発明は、以上のとおりの事情に鑑みてなされたものであり、外来性NAIP遺伝子の全cDNA配列を保有する超過排卵動物を提供することを目的としている。またこの発明は、上記動物の排卵数を人為的に促進させる方法を提供することを目的としている。

【0008】さらにこの発明は、ヒトを含めたナイーブ 動物個体(外来性遺伝子が導入されていない動物個体) の排卵数を人為的に増加させる方法を提供することを目 的としてもいる。

[0009]

【課題を解決するための手段】この発明は、上記の課題を解決するものとして、、プロモーター配列と配列番号1または2のDNA配列とを含むDNA断片を導入した分化全能性細胞を個体へと発生させたトランスジェニック非ヒト動物およびその子孫動物であって、体細胞染色体中に上記DNA断片を保有することを特徴とする超過排卵動物(請求項1)を提供する。

【0010】またこの発明は、プロモーター配列と配列番号1または2のDNA配列とを含むDNA断片を含有する組換えベクターにより形質転換した卵母細胞を卵巣中に導入された超過排卵動物(請求項5)を提供する。なおこれらの超過排卵動物においては、上記プロモーター配列が、性腺刺激ホルモンのレセプター遺伝子のプロモーター配列であること(請求項2および6)を好ましい態様としている。

【0011】さらにこの発明は、上記の超過排卵動物 (請求項1および5) の排卵を人為的に促進させる方法 であって、プロモーター配列の転写制御因子を動物に投 与し、配列番号3または4のアミノ酸配列を有するタンパク質を発現させることを特徴とする超過排卵方法 (請求項3および7)を提供する。さらにまたこの発明は、プロモーター配列が性腺刺激ホルモンのレセプター遺伝子のプロモーター配列である超過排卵動物 (請求項2および6) の排卵を人為的に促進させる方法であって、性 腺刺激ホルモンを動物に投与し、配列番号3または4のアミノ酸配列を有するタンパク質を発現させることを特 徴とする超過排卵方法 (請求項4および8)を提供する.

【0012】また、さらにこの発明は、ナイーブ動物個体の排卵を人為的に促進させる方法であって、タンパク質リン酸化酵素抑制因子またはスタウロスポリンもしくはそれらの誘導体を動物個体に投与することによって、配列番号3または4のアミノ酸配列を有するタンパク質を発現させることを特徴とする超過排卵方法(請求項9)を提供する。

【0013】以上の発明の他、この発明は、プロモーター配列と配列番号1または2のDNA配列とを含むDN

A断片を含有した組換えベクター(請求項10および12)、性腺刺激ホルモンのレセプター遺伝子のプロモーター配列と配列番号1または2のDNA配列とを含むDNA断片を含有した組換えベクター(請求項11および13)、上記のトランスジェニック非ヒト動物より単離された細胞(請求項14)、プロモーター配列と配列番号1または2のDNA配列とを含むDNA断片を含有する組換えベクターにより形質転換した卵母細胞(請求項16)をそれぞれ提供する。

【0014】以下、この発明の実施の形態について詳し 10 く説明する。

[0015]

【発明の実施の形態】この発明において、卵胞の退行・ 閉鎖抑制の手段として使用する遺伝子は、SMAの原因 遺伝子としてヒト第5染色体長腕13.1領域(5q1 3. 1) より単離されたNAIP遺伝子であり、その全 長cDNAは、配列番号1または配列番号2の塩基配列 を有している。このようなcDNAは、例えば配列番号 1または2の1部配列をプローブとして、既存の各種動 物由来のcDNAライブラリから単離することができ、 あるいは配列番号1または2の一部配列をプライマーと してPCR増幅して得ることができる。また、動物個体 に導入する c D N A は、配列番号 1 または 2 の D N A 配 列の全長でもよく、あるいは、そのコード領域部分でも よい。そして、この発明の超過排卵動物の作成において は、このcDNA配列と、その上流にプロモーター配列 を連結したDNA断片を調製し、このDNA断片を導入 した分化全能性細胞を個体へと発生させたトランスジェ ニック動物を作成する。

【0016】プロモーター配列は、このcDNA配列を 卵巣内で発現させるために、卵巣特異的に存在する物質 を転写制御因子とする遺伝子のプロモーター配列を用い るのが好ましく、特に、卵胞刺激ホルモンや黄体ホルモン等の性腺刺激ホルモンのレセプター遺伝子のプロモー ター配列が好ましい。あるいは、使用する動物種に内在 しない物質を転写制御因子とする外来遺伝子のプロモー ター配列を使用することもできる。この場合は、導入し たcDNAが自発的には発現せず、外部からその転写制 御因子を投与した場合にのみ発現させることができる。

【0017】例えば、この発明のトランスジェニック動物は以下の方法で作成することができる。プロモーター配列と配列番号1または2のDNA配列(またはその一部配列)とを、各々の解読枠を一致させて転結してDNA断片を調製し、このDNA断片を非ヒト哺乳動物の分化全能性細胞に導入する。対象となる動物は、例えばウシ、ブタ、ウマ、ヒツジ等の有用家畜であり、あるいはイヌやネコ、霊長類等の愛玩動物、実験用動物などである。また、分化全能性細胞としては、受精卵や初期胚のほか、ES細胞のような培養細胞を使用することができる。これらの分化全能性細胞へのDNA断片の注入は、

公知の方法、すなわち静電パルス法、リポソーム法、リ ン酸カルシウム法、マイクロインジェクション法等を用

いることができる。次に、DNA断片を注入した細胞を 仮親の卵管に移植し、個体まで発生した動物を出生させ て飼育する。そして、体細胞を取り出し、この細胞中の DNAをサザンブロット分析して導入したDNA断片の 存在を確認する。DNA断片の染色体への組み込みが確

認された個体を初代 (Founder)とすれば、この導入DNA断片はその子孫の50%に伝達され、この発明の超過排卵動物を継続的に生産することができる。

【0018】そして、このようにして作成した超過排卵動物は、その全細胞の染色体に導入DNA断片を有しており、プロモーター配列に特異的な転写制御因子の存在によってNAIP遺伝子のcDNAを発現させる。プロモーター配列が、卵胞刺激ホルモンのレセプター遺伝子のプロモーターである場合には、cDNAは、そのホレモンが存在する卵巣の顆粒膜細胞でのみ特異的に発現し、卵胞の退行・閉鎖を抑制する。もちろん、動物個体には内在性のNAIP遺伝子が存在して排卵数のコントロールを行っているが、この発明の超過排卵動物は、導入した外来性のNAIP遺伝子cDNAによって卵胞の退行・閉鎖抑制因子を多量に発現するため、ナイーブな動物に比べて多量の排卵が可能である。また、プロモーターに特異的な転写制御因子を投与することによって、超過排卵を人為的に制御することもできる。

【0019】一方、この発明の超過排卵動物は、動物個体の卵巣内に上記DNA断片を直接的に導入することによっても作成することができる。すなわち、DNA断片を組み込んだ組換えベクター(アデノウイルスベクター、レトロウイルスベクター等)を物理的な方法によって動物個体の卵巣に移植する。あるいは、上記組換えベクターによって形質転換した卵母細胞を卵巣に移植する。このような動物も、卵巣内においてNAIP遺伝子がナイーブ動物よりも多量発現し、超過排卵となる。また、NAIP遺伝子cDNAに連結したプロモーター配列の転写制御因子を投与することによって、その超過排卵をコントロールすることが可能である。

【0020】さらにまた、この発明は、ナイーブ動物個体の排卵を人為的に促進させる超過排卵方法も提供する。すなわち、全ての動物個体は、そのゲノム中にNAIP遺伝子を備えており、この内在性の遺伝子は、タンパク質リン酸化酵素抑制因子(例えば、免疫抑制剤FK506等)やスタウロスポリン(K252A等)、もしくはこれらの誘導体を全身投与することによって一過性に発現させることができる。これによって、ヒトを含めた動物個体において、排卵誘発剤等を用いることなく大量の排卵が可能となり、安全かつ確実な不妊療法が提供される。

【0021】次に、NAIP遺伝子が確かに卵胞の退行・閉鎖抑制に関与する遺伝子であることを確認した実験

結果を示し、この発明の有効性を説明する。

(1) 材料および方法

動物および性腺刺激ホルモン処理

ICR系雌マウス (日本クレア (株) より購入) を、明期12時間(5:00~17:00)、湿度ならびに温度を一定に調節した環境下で飼育し、3週令の時点で過排卵処理した。すなわち、5 I Uの妊馬血清性腺刺激ホルモン(pregnant mare serum gonadotropin, PMSG)を腹腔内投与し、48時間後に5 I Uのヒト絨毛性性腺刺激ホルモン(human chorionic gonadotropin, h CG)を同様に腹腔内投与した。

in situ hybridization

ブアン固定したマウス卵巣を脱水処埋したのち、パラフィン包埋し、厚さ $5\sim6~\mu$ m の切片に薄切後、シランコート処埋したスライドグラスに拾つた。乾燥させたのち、常法に従って脱パラフィンならび親水操作を行った組織切片を、0.3%Triton-X($2~\gamma$ 間)、 $0.2~\gamma$ HC1($2~\gamma$ 0分間)、 $20~\mu$ g/m1のプロテアーゼK($2~\gamma$ 0分間)で処埋した。さらに、 $4~\gamma$ 0の新鮮なパラフォルムアルデヒドに $5~\gamma$ 0分間浸漬して再固定させた後、 $0.2~\gamma$ 0グリシン中で $1~\gamma$ 1時間処理して残存アルデヒトを中和し、プレ・ハイブリダイゼーションとして50%フォルムアミドで $2~\gamma$ 1時間処理した。

【0022】ハイブリダイゼージョンに用いるNAIP のアンチセンスおよびセンスRNAプローブには、ヒト naip遺伝子 (Roy et al., Cell 80:167-178, 1995)の19 5 塩基から1263塩基までのBIR (baculoviral inhibit ion of apoptosis protein repeat)領域のBIR1、B IR2およびBIR3の一部を含む遺伝子断片(図2) をpBluscriptにサブクローニングしたベクターを用い て、ベーリンガーマンハイム社のジゴキシゲニン(Digo xigenin : DIG) - RNA labeling Kit を利用して in vitro転写させることによって生成したDIG標識R NAプローブを使用した。なお、このプローブについ て、IAP (inhibitor of apoptosis protein)関連遺伝 子であるマウスc-IAP1とのホモロジーは30~40%で あり、交差性が低いことが判っている。ハイブリダイゼ ーションは、50%フォルムアミド、10%硫酸デキストラ ン、1 X デンハルト溶液、100 μg/ml-本鎖サケ精子D NA、100 μg/ml大腸菌 t R NA、10 mMディチオステオー 40 ルの混台液に、予め65℃、5分間の処理で変性させたR NAプローブを加えた溶液を用いて、モイスチャーチャ ンバー中で50℃の条件下16~20時間行った。次に、未反 応のRNAプローブを組織標本から除くために以下の洗 浄操作を行った。まず、4 X S S C 中で20分間洗浄し、 20 µ g/mlのRNase中で37℃、30分間処理して洗浄し た後、さらに68℃の条件下2XSSCおよび 0.2XSS C中で各1時間洗浄した。

【0023】免疫組織化学反応による検出では、ベーリンガーマンハイム社のアルカリフォスファターゼ標識D

I G抗体を反応させた後、5-bromo-4-chloro-3-indoyl-phosphate-nitroblue tetrazolium (BCIP/NBT)を基質として4℃の条件下で発色させることでNAIP遺伝子のRNAの存在を示すシグナルを検出した。アポトーシスの組織化学的検出

マウス卵巣のブアン固定、パラフィン包埋、ならびに組織切片の作成については、前述の方法に準じた。形態学的なアポトーシスの検出は、染色体DNAのヌクレソーム単位での断片化を組織化学的に検出する方法であるTerminal Deoxynucleotidly Transferase (TdT)-mediated dUTP-biotin nick end labeling (TUNEL) 法を利用した。TUNELアッセイには、MEBSTAIN Kit (医学生物学研究所)を基本的に使用し、まず組織切片をプロテアーゼ処理した後、核における断片化DNAの3'0H末端部分にビオチン化dUTPを取り込ませた後、ストレプトアビジンーHRP (horseradish peroxidase)を加えて結合させ、基質としてDAB (diaminobenzidine)を使って発色させて断片化DNAを検出した。

顆粒膜細胞の単離および培養

3週令の I C R 幼若雌マウスより卵巣を摘出し、濾紙上 で脂肪組織や血液等を取り除き、イーグル最小必須培地 (minimum essential medium: MEM) で洗浄後に実験に 供試した。卵母細胞を含む顆粒膜細胞の細胞塊の単離お よび培養は、Eppig ら (Biol. Reprod., 41:268-276, 1 989)とHiranoら(J. Exp.Zool., 267:543-547, 1993) が 報告した方法にほぼ準じて行った。先ず、2mg/m1のコラ ゲナーゼ(細胞分散用、和光純薬)を添加したイーグル MEMに摘出した卵巣を浸漬し30分間処理した。洗浄 後、パスツールピペットでピペッティングを繰り返し て、卵母細胞を含む顆粒細胞の細胞塊を単離した。単離 した細胞塊は、寒天でコートした培養皿中の4mg/mlウシ 血清アルブミンおよび 2mMヒポキサンチンで培養した。 培養開始10日後に、これら卵母細胞を含む顆粒膜細胞の 細胞塊を100ng/mlの卵胞刺激ホルモン(fillicle-stimu lating hormone: FSH、シグマ)を含むイーグルME Mに移し、18時間後に卵母細胞を含む顆粒膜細胞の細胞 塊を採取し、NAIP遺伝子発現の解析に供した。 RNA抽出と逆転写酵素PCR (RT-PCR) および ノーザンブロット解析

卵巣からの全RNAの抽出は、AGPC (acid guanidium thiocyanate-phenol-chloroform)法 (Anal. Biochem. 162:156-159, 1987) によって行った。抽出した全RNAは、DNaseI処理することで混入したゲノムDNAを消化したのち、定量して実験に供した。

【0024】RT-PCR解析では、1 μg の全RNA からAMVXL RTase (宝酒造)によつてcDNA を合成し、その一部を使ってマウスnaipのcDNAを増幅した。増幅に使用したプライマーは、5'-CACAGGGGTGA AACTTGGGTTCAG-3'および5'-CACCTGTGGTTTCCATGGCTTCT GG-3'であり、反応条件は、熱変性94℃を5分間した

後、熱変性94℃を1分間、アニーリング60℃を1分間、 DNA合成72℃を2分間のサイクルを40回繰り退した。 増幅したRT-PCR産物は、2%アガロースゲルで電 気泳動して分離、検出した。

【0025】ノーザンブロット解析では、全RNAより Oligotex-dT<Super> (宝酒造)を使って精製した6~8 μg のポリ (A) + RNAを使用した。ホルムアミドの 存在下でアガロース電気泳動を分離した後、ナイロンフ ィルターである vbondN+ (アマシャム) にトランスフ ァーした。翌日、乾燥させたナイロンフィルターに紫外 線照射して、フィルター上のRNAを固定化した。プレ ハイブリダイゼーションおよひハイブリダイゼーション では、ExressHyb hybridization solution(クローンテ ック)を使用した。先ず、2~3時間のプレハイブリダ イゼーションの後、32 P-dCTP標識したマウスnnaip c DNAプローブを加えて一晩ハイブリダイゼーション を行った。なお、プローブに用いた遺伝子断片は、マウ スnaip遺伝子 (Robertson ら、未発表データ) の473 塩 基から1326塩基までのBIR1の一部とBIR2および BIR3を含む854 塩基の長さの遺伝子断片である(図 20 2)。やはり、IAP関連遺伝子であるマウスc-IAP 1とのホモロジーは30~40%であり、交差性が低いこと が判っている。ハイブリダイゼーションの洗浄は、2X SSCと0.05%SDSの水溶液中で室温、20分間を2 回、次に0.1 X S S C と 0.1 % D S D の 水溶液中で 68 ℃、20 分間を2回することで行った。洗浄したフィルタ ーは、イメージングプレート (フジ) でオートラジオグ ラフィーを行い、BAS-2000でシグナルを検出し、定 量解析を行った。

(2) 結果

卵巣内卵胞の発育に伴うNAIP遺伝子の発現

種々の週令の雌マウスより採取した卵巣におけるNAIP遺伝子の発現を、そのcDNAに対する特異的リボプローブを用いたin situ hybridization によって調べることで、卵巣内卵胞の発育に伴うNAIP遺伝子の発現を検討した。

【0026】出生直後の2日令マウス卵巣で観察される卵巣内卵胞像は、一層の扁平な顆粒膜細胞で卵母細胞が囲まれた原始卵胞、あるいは立方状になった顆粒膜細胞と卵母細胞、そして外周の基底膜で構成される一次卵胞 40の2つの卵胞像であるが、原始卵胞ではセンスおよびアンチセンスのリボプローブをハイブリダイズさせた場合とも差が見られなかった。一方、より発育した卵母細胞を持つ一次卵胞の顆粒膜細胞においてはアンチセンスリボプローブを使った際により強いシグナルが観察された(図3(A))。次に、性周期を規則的に繰り返す12週令の成熟したマウス卵巣においてNAIP遺伝子の発現を調べたところ、排卵直前の胞状卵胞であるグラーフ卵胞の顆粒膜細胞および卵母細胞を取り巻く卵丘細胞においてもNAIP遺伝子の発現が認められた(図3(B))。50

【0027】以上の結果から、NAIP遺伝子は、一次 卵胞から排卵直前のグラーフ卵胞までの顆粒膜細胞で発 現していることが確認された。

NAIP遺伝子の組織特異的発現

卵巣を含むマウスの各組織におけるNAIP遺伝子の発現をマウスnaipcDNAをプローブとしてノーザンブロット解析によって調べた。

【0028】NAIP遺伝子は、卵巣を含めマウス各組 織では2つの長さをもつ転写産物として発現している。 先ず、2μg のポリ (A) + RNAを結合させたマウスmu ltiple-tissue northernblot (MTN blot 、クローン テック)を使って、マウスの主な組織におけるNAIP 遺伝子の発現を検討したところ、牌臓、肺、肝臓、心臓 で強いシグナルが観られるものの、組織特異性はなく、 ほぼ全組織で発現が認められた(図4 (A))。一方、卵 巣での発現については、2日令、3週令、12週令、分娩 3日目、および18週令の種々の雌マウスより採取した卵 巣由来の8μg のポリ (A) RNAをブロットさせたフ ィルターを使ったところ、一次、二次卵胞や胞状卵胞の 割合が多い性周期を繰り返す成熟した12週令および18週 令の雌マウスで強いNAIP遺伝子の発現が観察され た。ただし、それぞれ原始卵胞や黄体が多くを占める2 日令や分娩3日めの雌マウスの卵巣においてはNAIP 遺伝子の発現は弱かった。

【0029】以上のことから、卵巣内におけるNAIP 遺伝子の発現は、卵胞の発達過程と相関関係にあること が確認された。

卵胞内におけるNAIP遺伝子発現の局在性

性腺刺激ホルモンで過排卵処埋を施した3週令の雌マウスの卵巣において、NAIP遺伝子の経時的な発現を調べた。卵胞刺激ホルモン(FSH)としての作用をもつPMSG投与48時間後の卵巣でのNAIP遺伝子の発現量は、投与前に比較して約1.6倍であり、さらに黄体ホルモン(Lutinating hormone, LH)作用を有するhCGの投与7時間後には、投与前と比較して約2.4倍強い発現が観察された。つぎに、卵巣より単離した卵母細胞を含む顆粒膜細胞の細胞塊におけるNAIP遺伝子の発現をRT-PCRによって検出したところ、卵母細胞では発現が認めらず、顆粒膜細胞でのみ発現していることが示された。さらに、PCRによる増幅のため絶対的な定量性はないが、性腺刺激ホルモンによってNAIP遺伝子の発現シグナルが強くなる傾向が観察された。

【0030】以上の結果から、NAIP遺伝子の発現は 卵胞の顆粒膜細胞に局在し、FSHなどの性腺刺激ホル モンによってその発現が増強されることが確認された。 卵胞閉鎖とNAIP遺伝子発現との関係

卵巣の連続組織切片上において、in situ hybridizatio n によって検出されるNAIP遺伝子の発現とTUNE L法によって認められるアポトーシスを比較検討した。その結果、図5に示したように、NAIP遺伝子が強く

発現している卵胞の顆粒膜細胞ではアポトーシスが観察されなかった。一方、卵母細胞の変形を特徴とする閉鎖卵胞では、細胞死した顆粒膜細胞が観察され、このような卵胞ではNAIP遺伝子の発現は、微弱であるか、またはほとんど観察されなかった。

【0031】以上のとおり、閉鎖した卵胞ではNAIP 遺伝子が発現していないことから、NAIP遺伝子は卵 巣内卵胞においてアポトーシス抑制因子として機能して いることが確認された。

[0032]

【発明の効果】以上詳しく説明したとおり、この発明によって、卵胞の退行・閉鎖を抑制するNAIP遺伝子を導入した超過排卵動物と、NAIP遺伝子の発現を制御

配列

することによって排卵を人為的に促進する方法が提供される。これによって、新たな不妊療法の開発が可能となり、有用動物の生産性も向上させることができる。

12

[0033]

【配列表】

配列番号:1 配列の長さ:5984 配列の型:核酸

鎖の数:二本鎖 10 トポロジー:直鎖状

配列の種類:cDNA to mRNA

起源

生物名:ヒト

ACAAAAGGTC CTGTGCTCAC CTGGGACCCT TCTGGACGTT GCCCTGTGTT CCTCTTCGCC 60 TGCCTGTTCA TCTACGACGA ACCCCGGGTA TTGACCCCAG ACAACAATGC CACTTCATAT 120 TGGGGACTTC GTCTGGGATT CCAAGGTGCA TTCATTGCAA AGTTCCTTAA ATATTTTCTC 180 ACTGCTTCCT ACTAAAGGAC GGACAGAGCA TTTGTTCTTC AGCCACATAC TTTCCTTCCA 240 CTGGCCAGCA TTCTCCTCTA TTAGACTAGA ACTGTGGATA AACCTCAGAA AATGGCCACC 300 CAGCAGAAAG CCTCTGACGA GAGGATCTCC CAGTTTGATC ACAATTTGCT GCCAGAGCTG 360 TCTGCTCTTC TGGGCCTAGA TGCAGTTCAG TTGGCAAAGG AACTAGAAGA AGAGGAGCAG 420 AAGGAGCGAG CAAAAATGCA GAAAGGCTAC AACTCTCAAA TGCGCAGTGA AGCAAAAAGG 480 TTANAGACTT TTGTGACTTA TGAGCCGTAC AGCTCATGGA TACCACAGGA GATGGCGGCC 540 GCTGGGTTTT ACTTCACTGG GGTAAAATCT GGGATTCAGT GCTTCTGCTG TAGCCTAATC 600 CTCTTTGGTG CCGGCCTCAC GAGACTCCCC ATAGAAGACC ACAAGAGGTT TCATCCAGAT 660 TGTGGGTTCC TTTTGAACAA GGATGTTGGT AACATTGCCA AGTACGACAT AAGGGTGAAG 720 AATCTGAAGA GCAGGCTGAG AGGAGGTAAA ATGAGGTACC AAGAAGAGGA GGCTAGACTT 780 GCATCCTTCA GGAACTGGCC ATTTTATGTC CAAGGGATAT CCCCTTGTGT GCTCTCAGAG 840 GCTGGCTTTG TCTTTACAGG TAAACAGGAC ACGGTACAGT GTTTTTCCTG TGGTGGATGT 900 TTAGGAAATT GGGAAGAAGG AGATGATCCT TGGAAGGAAC ATGCCAAATG GTTCCCCAAA 960 TGTGAATTTC TTCGGAGTAA GAAATCCTCA GAGGAAATTA CCCAGTATAT TCAAAGCTAC AAGGGATTTG TTGACATAAC GGGAGAACAT TTTGTGAATT CCTGGGTCCA GAGAGAATTA 1080 CCTATGGCAT CAGCTTATTG CAATGACAGC ATCTTTGCTT ACGAAGAACT ACGGCTGGAC 1140 TCTTTTAAGG ACTGGCCCCG GGAATCAGCT GTGGGAGTTG CAGCACTGGC CAAAGCAGGT 1200 CTTTTCTACA CAGGTATAAA GGACATCGTC CAGTGCTTTT CCTGTGGAGG GTGTTTAGAG 1260 AAATGGCAGG AAGGTGATGA CCCATTAGAC GATCACACCA GATGTTTTCC CAATTGTCCA TTTCTCCAAA ATATGAAGTC CTCTGCGGAA GTGACTCCAG ACCTTCAGAG CCGTGGTGAA 1380 CTTTGTGAAT TACTGGAAAC CACAAGTGAA AGCAATCTTG AAGATTCAAT AGCAGTTGGT 1440 CCTATACTGC CAGAAATGGC ACAGGGTGAA GCCCAGTGGT TTCAAGAGGC AAAGAATCTG 1500 AATGAGCAGC TGAGAGCAGC TTATACCAGC GCCAGTTTCC GCCACATGTC TTTGCTTGAT 1560 ATCTCTTCCG ATCTGGCCAC GGACCACTTG CTGGGCTGTG ATCTGTCTAT TGCTTCAAAA CACATCAGCA AACCTGTGCA AGAACCTCTG GTGCTGCCTG AGGTCTTTGG CAACTTGAAC 1680 TCTGTCATGT GTGTGGAGGG TGAAGCTGGA AGTGGAAAGA CGGTCCTCCT GAAGAAAATA 1740 GCTTTTCTGT GGGCATCTGG ATGCTGTCCC CTGTTAAACA GGTTCCAGCT GGTTTTCTAC 1800 CTCTCCCTTA GTTCCACCAG ACCAGACGAG GGGCTGGCCA GTATCATCTG TGACCAGCTC 1860 CTAGAGAAAG AAGGATCTGT TACTGAAATG TGCATGAGGA ACATTATCCA GCAGTTAAAG AATCAGGTCT TATTCCTTTT AGATGACTAC AAAGAAATAT GTTCAATCCC TCAAGTCATA 1980 GGAAAACTGA TTCAAAAAAA CCACTTATCC CGGACCTGCC TATTGATTGC TGTCCGTACA 2040

AACAGGGCCA GGGACATCCG CCGATACCTA GAGACCATTC TAGAGATCAA AGCATTTCCC 2100 TTTTATAATA CTGTCTGTAT ATTACGGAAG CTCTTTTCAC ATAATATGAC TCGTCTGCGA 2160

2220 AAGTTTATGG TTTACTTTGG AAAGAACCAA AGTTTGCAGA AGATACAGAA AACTCCTCTC TTTGTGGCGG CGATCTGTGC TCATTGGTTT CAGTATCCTT TTGACCCATC CTTTGATGAT GTGGCTGTTT TCAAGTCCTA TATGGAACGC CTTTCCTTAA GGAACAAAGC GACAGCTGAA 2340 ATTCTCAAAG CAACTGTGTC CTCCTGTGGT GAGCTGGCCT TGAAAGGGTT TTTTTCATGT TGCTTTGAGT TTAATGATGA TGATCTCGCA GAAGCAGGGG TTGATGAAGA TGAAGATCTA ACCATGTGCT TGATGAGCAA ATTTACAGCC CAGAGACTAA GACCATTCTA CCGGTTTTTA 2520 AGTCCTGCCT TCCAAGAATT TCTTGCGGGG ATGAGGCTGA TTGAACTCCT GGATTCAGAT 2580 AGGCAGGAAC ATCAAGATTT GGGACTGTAT CATTTGAAAC AAATCAACTC ACCCATGATG 2640 ACTGTAAGCG CCTACAACAA TTTTTTGAAC TATGTCTCCA GCCTCCCTTC AACAAAAGCA 2700 GGGCCCAAAA TTGTGTCTCA TTTGCTCCAT TTAGTGGATA ACAAAGAGTC ATTGGAGAAT 2760 ATATCTGAAA ATGATGACTA CTTAAAGCAC CAGCCAGAAA TTTCACTGCA GATGCAGTTA 2820 CTTAGGGGAT TGTGGCAAAT TTGTCCACAA GCTTACTTTT CAATGGTTTC AGAACATTTA 2880 CTGGTTCTTG CCCTGAAAAC TGCTTATCAA AGCAACACTG TTGCTGCGTG TTCTCCATTT 2940 GTTTTGCAAT TCCTTCAAGG GAGAACACTG ACTTTGGGTG CGCTTAACTT ACAGTACTTT 3000 TTCGACCACC CAGAAAGCTT GTCATTGTTG AGGAGCATCC ACTTCCCAAT ACGAGGAAAT 3060 AAGACATCAC CCAGAGCACA TTTTTCAGTT CTGGAAACAT GTTTTGACAA ATCACAGGTG 3120 CCAACTATAG ATCAGGACTA TGCTTCTGCC TTTGAACCTA TGAATGAATG GGAGCGAAAT 3180 TTAGCTGAAA AAGAGGATAA TGTAAAGAGC TATATGGATA TGCAGCGCAG GGCATCACCA 3240 GACCTTAGTA CTGGCTATTG GAAACTTTCT CCAAAGCAGT ACAAGATTCC CTGTCTAGAA 3300 GTCGATGTGA ATGATATTGA TGTTGTAGGC CAGGATATGC TTGAGATTCT AATGACAGTT 3360 TTCTCAGCTT CACAGCGCAT CGAACTCCAT TTAAACCACA GCAGAGGCTT TATAGAAAGC 3420 ATCCGCCCAG CTCTTGAGCT GTCTAAGGCC TCTGTCACCA AGTGCTCCAT AAGCAAGTTG 3480 GAACTCAGCG CAGCCGAACA GGAACTGCTT CTCACCCTGC CTTCCCTGGA ATCTCTTGAA 3540 GTCTCAGGGA CAATCCAGTC ACAAGACCAA ATCTTTCCTA ATCTGGATAA GTTCCTGTGC 3600 CTGAAAGAAC TGTCTGTGGA TCTGGAGGGC AATATAAATG TTTTTTCAGT CATTCCTGAA 3660 GAATTTCCAA ACTTCCACCA TATGGAGAAA TTATTGATCC AAATTTCAGC TGAGTATGAT 3720 CCTTCCAAAC TAGTAAAATT AATTCAAAAT TCTCCAAACC TTCATGTTTT CCATCTGAAG 3780 TGTAACTTCT TTTCGGATTT TGGGTCTCTC ATGACTATGC TTGTTTCCTG TAAGAAACTC 3840 ACAGAAATTA AGTTTTCGGA TTCATTTTT CAAGCCGTCC CATTTGTTGC CAGTTTGCCA 3900 AATTTTATTT CTCTGAAGAT ATTAAATCTT GAAGGCCAGC AATTTCCTGA TGAGGAAACA 3960 TCAGAAAAAT TTGCCTACAT TTTAGGTTCT CTTAGTAACC TGGAAGAATT GATCCTTCCT 4020 ACTGGGGATG GAATTTATCG AGTGGCCAAA CTGATCATCC AGCAGTGTCA GCAGCTTCAT 4080 TGTCTCCGAG TCCTCTCATT TTTCAAGACT TTGAATGATG ACAGCGTGGT GGAAATTGCC 4140 AAAGTAGCAA TCAGTGGAGG TTTCCAGAAA CTTGAGAACC TAAAGCTTTC AATCAATCAC 4200 AAGATTACAG AGGAAGGATA CAGAAATTTC TTTCAAGCAC TGGACAACAT GCCAAACTTG CAGGAGTTGG ACATCTCCAG GCATTTCACA GAGTGTATCA AAGCTCAGGC CACAACAGTC 4320 AAGTCTTTGA GTCAATGTGT GTTACGACTA CCAAGGCTCA TTAGACTGAA CATGTTAAGT 4380 TGGCTCTTGG ATGCAGATGA TATTGCATTG CTTAATGTCA TGAAAGAAAG ACATCCTCAA 4440 TCTAAGTACT TAACTATTCT CCAGAAATGG ATACTGCCGT TCTCTCCAAT CATTCAGAAA 4500 TAAAAGATTC AGCTAAAAAC TGCTGAATCA ATAATTTGTC TTGGGGCATA TTGAGGATGT 4560 AAAAAAAGTT GTTGATTAAT GCTAAAAACC AAATTATCCA AAATTATTTT ATTAAATATT 4620 4680 GCATACTCAC CACCAAGCTC AAGAAATAAA TCATCACCAA TACCTTTGAG GTCCCTGAGT 4740 AATCCACCCC AGCTAAAGGC AAACCCTTCA ATCAAGTTTA TACAGCAAAC CCTCCATTGT 4800 CCATGGTCAA CAGGGAAGGG GTTGGGGACA GGTCTGCCAA TCTATCTAAA AGCCACAATA 4860 TGGAAGAAGT ATTCAATTTA TATAATAAAT GGCTAACTTA ACGGTTGAAT CACTTTCATA 4920 CATGGATGAA ACGGGTTTAA CACAGGATCC ACATGAATCT TCTGTGGGCC AAAATATGTT 4980 CCTTAATCCT TGTAGAACCT GTCTTCTATA TTGAACTAGC TTTGGTACAG TAGAGTTAAC 5040 TTACTTTCCA TTTATCCACT GCCAATATAA AGAGGAAACA GGGGTTAGGG AAAAATGACT 5100 TCATTCCAGA GGCTTCTCAG AGTTCAACAT ATGCTATAAT TTAGAATTTT CTTATGAATC 5160

CACTCTACTT	GGGTAGAAAA	TATTTTATCT	CTAGTGATTG	CATATTATTT	CCATATCATA	5220
${\tt GTATTTCATA}$	${\tt GTATTATATT}$	TGATATGAGT	$GTCT\LambdaT\LambdaTC\Lambda$	ATGTCAGTGT	CCAGAATTTC	5280
GTTCCTACCA	${\tt GTTGAGTAGT}$	TTTCTGAACG	GCCAGAAGAC	CATTCGAAAT	TCATGATACT	5340
${\tt ACTATAAGTT}$	${\tt GGTAAACAAC}$	${\tt CATACTTTA}$	TCCTCATTTT	TATTCTCACT	AAGAAAAAAG	5400
${\sf TCAACTCCCC}$	${\tt TCCCCTTGCC}$	${\tt CAAGTATGAA}$	${\tt ATATAGGGAC}$	${\tt AGTATGTATG}$	GTGTGGTCTC	5460
${\tt ATTTGTTTAG}$	AAAACCACTT	${\tt ATGACTGGGT}$	${\tt GCGGTGGCTC}$	ACACCTGTAA	TCCCAGCACT	5520
${\tt TTGGGAGGCT}$	${\sf GAGGCGGGCG}$	${\tt AATCATTTGA}$	${\tt GGTGAGGAGT}$	TCGAGACCGG	CCTGGCCAGC	5580
${\tt ATGGTGAAAC}$	CCCATTTTTG	${\tt CTAAAGGTAC}$	AAAAATTAGC	${\tt CAGGTGTGGT}$	GGCACATGCC	5640
${\tt TGTGGTCCCA}$	${\tt GCCACTGGGG}$	${\tt CGGCTGAGAC}$	${\tt GCAGGACTTG}$	${\tt CTTGAACCCG}$	GGAGGCAGAG	5700
${\tt GTTGCAGTGA}$	${\tt GCCGAGATGG}$	$CGCC \Lambda CTGC \Lambda \\$	${\tt TTCCAGCCTG}$	GGCAACAGAG	CAAGACCCTG	5760
${\tt TCTGTTTCAA}$	${\tt AACAAAAAAC}$	${\tt AAAACCACTT}$	ATATTGCTAG	${\tt CTACATTAAG}$	AATTTCTGAA	5820
${\tt TATGTTACTG}$	${\tt AGCTTGCTTG}$	${\tt TGGTAACCAT}$	${\bf TTATAATATC}$	${\sf AGAAAGTATA}$	TGTACACCAA	5880
${\tt AACATGTTGA}$	ACATCCATGT	${\tt TGTACAACTG}$	${\tt AAATATAAAT}$	AATTTTGTCA	ATTATACCTA	5940
AATAAAACTG	GAAAAAAAAAA	${\tt AAAAAAAAAA}$	${\sf AAAAAAAAA}$	AAAA		5984

配列番号: 2 配列の長さ: 5366 配列の型: 核酸 鎖の数: 二本鎖 トポロジー:直鎖状 配列の種類:cDNA to mRNA

起源

生物名:ヒト

配列

ACAAAAGGTC	CTGTGCTCAC	CTGGGACCCT	TCTGGACGTT	GCCCTGTGTT	CCTCTTCGCC	60
TGCCTGTTCA	TCTACGACGA	ACCCCGGGTA	TTGACCCCAG	ACAACAATGC	CACTTCATAT	120
${\tt TGGGGACTTC}$	${\tt GTCTGGGATT}$	CCAAGGTGCA	TTCATTGCAA	AGTTCCTTAA	ATATTTTCTC	180
ACTGCTTCCT	${\tt ACTAAAGGAC}$	GGACAGAGCA	TTTGTTCTTC	AGCCACATAC	TTTCCTTCCA	240
${\tt CTGGCCAGCA}$	TTCTCCTCTA	TTAGACTAGA	ACTGTGGATA	AACCTCAGAA	AATGGCCACC	300
CAGCAGAAAG	CCTCTGACGA	GAGGATCTCC	${\sf CAGTTTGATC}$	ACAATTTGCT	GCCAGAGCTG	360
TCTGCTCTTC	TGGGCCTAGA	TGCAGTTCAG	${\tt TTGGCAAAGG}$	AACTAGAAGA	AGAGGAGCAG	420
AAGGAGCGAG	CAAAAATGCA	GAAAGGCTAC	AACTCTCAAA	TGCGCAGTGA	AGCAAAAAGG	480
TTAAAGACTT	TTGTGACTTA	${\sf TGAGCCGTAC}$	${\tt AGCTCATGGA}$	TACCACAGGA	GATGGCGGCC	540
GCTGGGTTTT	ACTTCACTGG	GGTAAAATCT	GGGATTCAGT	GCTTCTGCTG	TAGCCTAATC	600
$\mathtt{CTCTTTGGTG}$	CCGGCCTCAC	GAGACTCCCC	ATAGAAGACC	ACAAGAGGTT	TCATCCAGAT	660
${\tt TGTGGGTTCC}$	TTTTGAACAA	${\tt GGATGTTGGT}$	AACATTGCCA	$\Lambda GTACGACAT$	AAGGGTGAAG	720
AATCTGAAGA	GCAGGCTGAG	AGGAGGTAAA	${\tt ATGAGGTACC}$	AAGAAGAGGA	GGCTAGACTT	780
GCATCCTTCA	${\tt GGAACTGGCC}$	${\tt ATTTTATGTC}$	${\sf CAAGGGATAT}$	${\tt CCCCTTGTGT}$	GCTCTCAGAG	840
${\tt GCTGGCTTTG}$	${\tt TCTTTACAGG}$	TAAACAGGAC	${\tt ACGGTACAGT}$	GTTTTTCCTG	TGGTGGATGT	900
${\tt TTAGGAAATT}$	${\tt GGGAAGAAGG}$	AGATGATCCT	${\bf TGGAAGGAAC}$	${\tt ATGCCAAATG}$	GTTCCCCAAA	960
${\tt TGTGAATTTC}$	TTCGGAGTAA	GAAATCCTCA	${\sf GAGGAAATTA}$	${\tt CCCAGTATAT}$	TCAAAGCTAC	1020
${\tt AAGGGATTTG}$	${\tt TTGACATAAC}$	${\tt GGGAGAACAT}$	${\tt TTTGTGAATT}$	${\tt CCTGGGTCCA}$	GAGAGAATTA	1080
CCTATGGCAT	${\sf CAGCTTATTG}$	${\sf CAATGACAGC}$	ATCTTTGCTT	ACGAAGAACT	ACGGCTGGAC	1140
${\tt TCTTTTAAGG}$	ACTGGCCCCG	${\sf GGAATCAGCT}$	${\tt GTGGGAGTTG}$	${\sf CAGCACTGGC}$	CAAAGCAGGT	1200
${\tt CTTTTCTACA}$	${\sf CAGGTATAAA}$	${\tt GGACATCGTC}$	${\tt CAGTGCTTTT}$	${\tt CCTGTGGAGG}$	GTGTTTAGAG	1260
${\tt AAATGGCAGG}$	${\tt AAGGTGATGA}$	${\tt CCCATTAGAC}$	${\sf GATCACACCA}$	${\tt GATGTTTTCC}$	CAATTGTCCA	1320
${\tt TTTCTCCAAA}$	${\tt ATATGAAGTC}$	CTCTGCGGAA	${\tt GTGACTCCAG}$	${\tt ACCTTCAGAG}$	CCGTGGTGAA	1380
${\tt CTTTGTGAAT}$	${\sf TACTGGAAAC}$	${\sf CACAAGTGAA}$	${\tt AGCAATCTTG}$	${\tt AAGATTCAAT}$	AGCAGTTGGT	1440
${\tt CCTATAGTGC}$	${\sf CAGAAATGGC}$	${\tt ACAGGGTGAA}$	${\tt GCCCAGTGGT}$	${\tt TTCAAGAGGC}$	AAAGAATCTG	1500
${\tt AATGAGCAGC}$	${\tt TGAGAGCAGC}$	${\tt TTATACCAGC}$	${\tt GCCAGTTTCC}$	${\tt GCCACATGTC}$	TTTGCTTGAT	1560
${\tt ATCTCTTCCG}$	${\tt ATCTGGCCAC}$	${\tt GGACCACTTG}$	${\tt CTGGGCTGTG}$	${\tt ATCTGTCTAT}$	TGCTTCAAAA	1620
CACATCAGCA	$\Lambda\Lambda CCTGTGC\Lambda$	${\tt AGAACCTCTG}$	${\tt GTGCTGCCTG}$	$\Lambda GGTCTTTGG$	СЛЛСТТБЛЛС	1680
${\tt TCTGTCATGT}$	${\tt GTGTGGAGGG}$	TGAAGCTGGA	${\tt AGTGGAAAGA}$	${\tt CGGTCCTCCT}$	GAAGAAAATA	1740
$\tt GCTTTTCTGT$	${\tt GGGCATCTGG}$	ATGCTGTCCC	${\tt CTGTTAAACA}$	${\tt GGTTCCAGCT}$	GGTTTTCTAC	1800
CTCTCCCTTA	GTTCCACCAG	ACCAGACGAG	GGGCTGGCCA	${\tt GTATCATCTG}$	TGACCAGCTC	1860

17 CTAGAGAAAG AAGGATCTGT TACTGAAATG TGCATGAGGA ACATTATCCA GCAGTTAAAG 1920 AATCAGGTCT TATTCCTTTT AGATGACTAC AAAGAAATAT GTTCAATCCC TCAAGTCATA GGAAAACTGA TTCAAAAAAA CCACTTATCC CGGACCTGCC TATTGATTGC TGTCCGTACA 2040 AACAGGGCCA GGGACATCCG CCGATACCTA GAGACCATTC TAGAGATCAA AGCATTTCCC 2100 TTTTATAATA CTGTCTGTAT ATTACGGAAG CTCTTTTCAC ATAATATGAC TCGTCTGCGA 2160 AAGTTTATGG TTTACTTTGG AAAGAACCAA AGTTTGCAGA AGATACAGAA AACTCCTCTC 2220 TTTGTGGCGG CGATCTGTGC TCATTGGTTT CAGTATCCTT TTGACCCATC CTTTGATGAT 2280 GTGGCTGTTT TCAAGTCCTA TATGGAACGC CTTTCCTTAA GGAACAAAGC GACAGCTGAA 2340 ATTCTCAAAG CAACTGTGTC CTCCTGTGGT GAGCTGGCCT TGAAAGGGTT TTTTTCATGT 2400 TGCTTTGAGT TTAATGATGA TGATCTCGCA GAAGCAGGGG TTGATGAAGA TGAAGATCTA 2460 ACCATGTGCT TGATGAGCAA ATTTACAGCC CAGAGACTAA GACCATTCTA CCGGTTTTTA AGTCCTGCCT TCCAAGAATT TCTTGCGGGG ATGAGGCTGA TTGAACTCCT GGATTCAGAT 2580 AGGCAGGAAC ATCAAGATTT GGGACTGTAT CATTTGAAAC AAATCAACTC ACCCATGATG 2640 ACTGTAAGCG CCTACAACAA TTTTTTGAAC TATGTCTCCA GCCTCCCTTC AACAAAAGCA 2700 GGGCCCAAAA TTGTGTCTCA TTTGCTCCAT TTAGTGGATA ACAAAGAGTC ATTGGAGAAT 2760 ATATCTGAAA ATGATGACTA CTTAAAGCAC CAGCCAGAAA TTTCACTGCA GATGCAGTTA CTTAGGGGAT TGTGGCAAAT TTGTCCACAA GCTTACTTTT CAATGGTTTC AGAACATTTA 2880 CTGGTTCTTG CCCTGAAAAC TGCTTATCAA AGCAACACTG TTGCTGCGTG TTCTCCATTT 2940 GTTTTGCAAT TCCTTCAAGG GAGAACACTG ACTTTGGGTG CGCTTAACTT ACAGTACTTT 3000 TTCGACCACC CAGAAAGCTT GTCATTGTTG AGGAGCATCC ACTTCCCAAT ACGAGGAAAT 3060 AAGACATCAC CCAGAGCACA TTTTTCAGTT CTGGAAACAT GTTTTGACAA ATCACAGGTG 3120 CCAACTATAG ATCAGGACTA TGCTTCTGCC TTTGAACCTA TGAATGAATG GGAGCGAAAT 3180 TTAGCTGAAA AAGAGGATAA TGTAAAGAGC TATATGGATA TGCAGCGCAG GGCATCACCA 3240 GACCTTAGTA CTGGCTATTG GAAACTTTCT CCAAAGCAGT ACAAGATTCC CTGTCTAGAA 3300 GTCGATGTGA ATGATATTGA TGTTGTAGGC CAGGATATGC TTGAGATTCT AATGACAGTT 3360 TTCTCAGCTT CACAGCGCAT CGAACTCCAT TTAAACCACA GCAGAGGCTT TATAGAAAGC 3420 ATCCGCCCAG CTCTTGAGCT GTCTAAGGCC TCTGTCACCA AGTGCTCCAT AAGCAAGTTG 3480 GAACTCAGCG CAGCCGAACA GGAACTGCTT CTCACCCTGC CTTCCCTGGA ATCTCTTGAA 3540 GTCTCAGGGA CAATCCAGTC ACAAGACCAA ATCTTTCCTA ATCTGGATAA GTTCCTGTGC 3600 CTGAAAGAAC TGTCTGTGGA TCTGGAGGGC AATATAAATG TTTTTTCAGT CATTCCTGAA 3660 GAATTTCCAA ACTTCCACCA TATGGAGAAA TTATTGATCC AAATTTCAGC TGAGTATGAT CCTTCCAAAC TAGTAAAATT AATTCAAAAT TCTCCAAACC TTCATGTTTT CCATCTGAAG 3780 TGTAACTTCT TTTCGGATTT TGGGTCTCTC ATGACTATGC TTGTTTCCTG TAAGAAACTC 3840 ACAGAAATTA AGTTTTCGGA TTCATTTTT CAAGCCGTCC CATTTGTTGC CAGTTTGCCA 3900 AATTTTATTT CTCTGAAGAT ATTAAATCTT GAAGGCCAGC AATTTCCTGA TGAGGAAACA 3960 TCAGAAAAAT TTGCCTACAT TTTAGGTTCT CTTAGTAACC TGGAAGAATT GATCCTTCCT 4020 ACTGGGGATG GAATTTATCG AGTGGCCAAA CTGATCATCC AGCAGTGTCA GCAGCTTCAT 4080 TGTCTCCGAG TCCTCTCATT TTTCAAGACT TTGAATGATG ACAGCGTGGT GGAAATTGGT 4140 GAGCTAGTGT TTCAGCTTGC ATGGAAGCCA GTGGTATAGC CAAGCTTTCT GCTGCAACAT 4200 GTCTATGTAA ACATTTGCCC CTCTAGAAAT TTTCAACCCG CTTCCTCATT TTCACTATCA 4260 TACTGTTCCT TCTAGTGTCC TTCTGTGGAT TTAGGCGCAT TCTGGTCAGA TTTGGAAGTA CAAAAAGGTC TCCCATTTGT GGATATACAA GCCCTCAAAT CTGCGTTCTT GCCACCTGGT 4380 GTTTTAGACA CCTGGCCACA TACTCTCCTA AGTACTCCTT TTTAAAACTG AAGATGAATA 4440 TACACACAGA AAAGTACAAA AATCATGTGT ACTGCTCACT GAATTTTATT TTCTTATTTT 4500 CTTCTTTTT TTTTTTTGA GACAGAGTTT CGCTCGTGTT GCCCAGGCTG GAGTACAATG 4560

GCACGATCTC GGGTCACTGC AAACTCTGCC TCCTGGGTTC AAGCGATTCT CCTGCCTCAG

CCTCCCAAGT AGCTAGGATT ACAGGTGAAC GCCACCACAC CTGGCTAATT TTGTATTTTT

AGTAAACACA GGGTTTCACC ATGTTGGCCA GGCTAGTCTC GAACTCCTGA CCTCAAGTGA

GCCACAGTGC CTGGCCTGAG GAACTGAGAT TTCTGTCGAG ACCTGAAGGG AGAATGGCCC

AGGCATAGTT GGTAGAGGAG GAATTGAGAC ATCATTTCAA ACAGAGGTAA TCACTTGTGT

4620

4680

4740

CATAGCCTGG	${\tt AGTTAAAGAG}$	AACCAGATAT	ATTTGAAGAA	${\tt CTTGGGGGAA}$	AAAAAGGAAT	4920	
${\tt GTCTGGAGCA}$	$\Lambda G \Lambda G G C \Lambda G G \Lambda$	${\tt GTGAGTTGTG}$	AGAAGAAGAC	${\tt TGGAGAGGAA}$	AGTAAAAGCC	4980	
${\sf CAATTGGAGA}$	${\tt GGCTTTGTCG}$	${\tt GGTGTGTTAC}$	${\tt AAGGGCTGGA}$	${\tt TCTCATTTTC}$	TTACTGCTCA	5040	
${\tt GCACTGTTAT}$	TTTACGTTAT	${\bf TTAAAACAGC}$	${\tt TGGGAGCGGT}$	GGCTCAAGCT	TGTAATCCCA	5100	
${\tt GCACTTTGGG}$	AGGCCGAGGC	${\tt GGATGGATCA}$	${\sf CGAGGTCAGG}$	${\sf AGATCGAGAC}$	CATCCTGGCT	5160	
AACATGGTGA	AACCCCGTCT	${\sf CTACTAAAAA}$	${\tt TACAAAAAAT}$	TAGCCAGGCG	TGATGGCGGG	5220	
CACCTGTAGT	CCCAGCTACT	${\tt CGGGAGGCTG}$	${\tt AGGCAGGAGA}$	${\bf ATGGTGTGAA}$	CCCGGGAGGT	5280	
GGAGCTTGAA	${\tt GTGAGCCAAG}$	ATCATGCCAC	${\tt TGCACTCCAG}$	${\tt CCTGGGCAAC}$	AGAACGAGAC	5340	
TCCGTCTCAA	${\tt AAAAAAAAAA}$	CAAAAA				5366	

配列番号:3 配列の長さ:1404 10 トポロジー:直鎖状配列の種類:タンパク質

配列の型:アミノ酸

配列

团列															
Met	Ala	Thr	Gln	Gln	Lys	Ala	Ser	Asp	Glu	Arg	Ile	Ser	Gln	Phe	Asp
1				5					10					15	
His	Asn	Leu	Leu 20	Pro	Glu	Leu	Ser	Ala 25	Leu	Leu	Gly	Leu	Asp 30	Ala	Val
Gln	Leu	Ala 35	Lys	Glu	Leu	Glu	G1u 40	G1u	Glu	Gln	Lys	G1u 45	Arg	Ala	Lys
Met	G1n 50	Lys	Gly	Tyr	Asn	Ser 55	Gln	Met	Arg	Ser	G1u 60	Ala	Lys	Arg	Leu
Lys 65	Thr	Phe	Val	Thr	Tyr 70	Glu	Pro	Tyr	Ser	Ser 75	Trp	He	Pro	Gln	G1u 80
Met	Ala	Ala	Ala	G1y 85	Phe	Tyr	Phe	Thr	G1y 90	Val	Lys	Ser	Gly	I1e 95	Gln
Cys	Phe	Cys	Cys 100	Ser	Leu	Ile	Leu	Phe 105	Gly	Ala	Gly	Leu	Thr 110	Arg	Leu
Pro	lle	G1u 115	Asp	His	Lys	Arg	Phe 120	His	Pro	Asp	Cys	Gly 125	Phe	Leu	Leu
Asn	Lys 130	Asp	Val	Gly	Asn	I1e 135	Ala	Lys	Tyr	Asp	I le 140	Arg	Val	Lys	Asņ
Leu	Lys	Ser	Arg	Leu	Arg	Gly	Gly	Lys	Met	Arg	Tyr	Gln	Glu	Glu	Glu
145					150					155					160
Ala	Arg	Leu	Ala	Ser 165	Phe	Arg	Asn	Trp	Pro 170	Phe	Tyr	Val	Gln	G1y 175	He
Ser	Pro	Cys	Va1 180	Leu	Ser	Glu	Ala	Gly 185	Phe	Val	Phe	Thr	G1y 190	Lys	G1n
Asp	Thr	Val 195	Gln	Cys	Phe	Ser	Cys 200	Gly	Gly	Cys	Leu	G1y 205	Asn	Trp	G1u
Glu	Gly 210	Asp	Asp	Pro	Trp	Lys 215	Glu	His	Ala	Lys	Trp 220	Phe	Pro	Lys	Cys
G1u 225	Phe	Leu	Arg	Ser	Lys 230	Lys	Ser	Ser	Glu	G1u 235	He	Thr	Gln	Tyr	I 1e 240
Gln	Ser	Tyr	Lys	G1y 245	Phe	Val	Asp	He	Thr 250	Gly	Glu	His	Phe	Val 255	Asn
Ser	Trp	Val	G1n 260	Arg	Glu	Leu	Pro	Met 265	Ala	Ser	Ala	Tyr	Cys 270	Asn	Asp
Ser	Ile	Phe 275	Ala	Tyr	Glu	Glu	Leu 280	Arg	Leu	Asp	Ser	Phe 285	Lys	Asp	Trp
Pro	Arg	Glu	Ser	Ala	Val	Gly	Val	Ala	Ala	Leu	Ala	Lys	Ala	Gly	Leu

	290					295					300				
Phc	Tyr	Thr	Gly	He	Lys	٨sp	He	Val	Gln	Cys	Phe	Ser	Cys	Gly	Gly
305					310					315					320
Cys	Leu	G1u	Lys	Trp 325	Gln	Glu	Gly	Asp	Asp 330	Pro	Leu	Asp	Asp	His	Thr
1	Curc	Dho	Dro		Cuc	Dro	Dho	Lou		Acn	Wat	Luc	Sor		۸1a
Arg	cys	rne	340	ASN	cys	rro	rne	345	GIII	ASII	Mer	Lys	Ser 350	ser	міа
Glu	Val	Thr 355	Pro	Asp	Leu	Gln	Ser 360	Arg	Gly	Glu	Leu	Cys 365	Glu	Leu	Leu
Glu	Thr 370	Thr	Ser	Glu	Ser	Λsn 375	Leu	Glu	Asp	Ser	I1e 380	Λla	Val	Gly	Pro
He		Pro	Glu	Met	Ala	G1n	G1v	Glu	Ala	Gln	Trp	Phe	Gln	Glu	Ala
385					390		5			395					400
	Asn	Leu	Asn			Leu	Arg	Ala			Thr	Ser	Ala		
	••	.,		405					410				т.	415	** .
Arg	His	Met	Ser 420	Leu	Leu	Asp	He	Ser 425	Ser	Asp	Leu	Ala	Thr 430	Asp	HIS
Leu	Leu	G1y 435	Cys	Asp	Leu	Ser	Ile 440	Ala	Ser	Lys	His	I 1e 445	Ser	Lys	Pro
Val	C1n		Pro	ا ما	Va1	Len		Clu	Val	Phe	Glv		Leu	Asn	Ser
Vai	450	Giu	110	LCu	V & 1	455	110	Giu	141	1110	460	71311	DCu	11.311	JC.
Val	Met	Cys	Val	Glu	Gly	Glu	Ala	Gly	Ser	Gly	Lys	Thr	Val	Leu	Leu
465					470					475					480
Lys	Lys	Ile	Ala	Phe 485	Leu	Trp	Ala	Ser	Gly 490	Cys	Cys	Pro	Leu	Leu 495	Asn
Arg	Phe	Gln	Leu 500	Val	Phe	Tyr	Leu	Ser 505	Leu	Ser	Ser	Thr	Arg 510	Pro	Asp
Glu	Gly	Leu 515		Ser	He	He	Cys 520	Asp	Gln	Leu	Leu	G1u 525	Lys	Glu	Gly
Sar	Val		Clu	Mar	Cue	Mot		Acn	Ιle	Ile	Cln		Leu	lvs	Asn
	530					535					540				
	Val	Leu	Phe	Leu		Asp	Asp	Tyr	Lys		He	Cys	Ser	He	
545					550	_	_			555	_	_		-	560
Gln	Val	He	Gly	Lys 565	Leu	He	Gln	Lys	Asn 570	His	Leu	Ser	Arg	Thr 575	Cys
Leu	Leu	He	A1a 580	Val	Arg	Thr	Asn	Arg 585	Ala	Arg	Asp	He	Arg 590	Arg	Tyr
Leu	Glu	Thr 595	Ile	Leu	Glu	Ile	Lys 600	Ala	Phe	Pro	Phe	Tyr 605	Asn	Thr	Val
Cvc	I 1 o		1.50	Lvc	Len	Pho		Hic	Aen	Mot	Thr		Leu	Aro	Lvs
cys	610	LCU	лід	Lys	Leu	615	561	1113	ASII	MCL	620	Al g	bcu	B	Lys
Phe	Met	Val	Tyr	Phe	Gly	Lys	Asn	Gln	Ser	Leu	Gln	Lys	He	Gln	Lys
625					630					635					640
Thr	Pro	Leu	Phe	Val 645	Ala	Ala	He	Cys	A1a 650	His	Trp	Phe	Gln	Tyr 655	Pro
Phe	Asp	Pro	Ser 660		Asp	Asp	Val	A1a 665		Phe	Lys	Ser	Tyr 670		Glu
Arg	Leu	Ser		Arg	Asn	Lys		Thr	Ala	Glu	He		Lys	Ala	Thr
		675		_	_	_	680			_	-	685		•	
Val	Ser	Ser	Cvs	Glv	Glu	Leu	Ala	1.611	Lvs	Glv	Phe	Phe	Ser	Uvs	UVS

	690					695					700				
Phe	Glu	Phe	Λsn	Asp	Λsp	Asp	Leu	Λla	Glu	Λla	Gly	Val	Λsp	Glu	Λsp
705					710					715					720
Glu	Asp	Leu	Thr	Mer	Cvs	Leu	Met	Ser	Lvs	Phe	Thr	Ala	Gln	Are	Leu
				725	-5-				730					735	
Ara	Dro	Pho	Tyr		Pho	Lou	Sor	Pro		Pho	Cln	Glu	Pho		Δla
AI g	FIO	rne		vi B	rne	Leu	Sei		піа	THE	GIII	Giu		Leu	піа
٥.			740		0.			745				C 1	750	** .	٠,
Gly	Met		Leu	He	Glu	Leu		Asp	Ser	Asp	Arg		Glu	HIS	Gin
		755					760					765			
Asp	Leu	Gly	Leu	Tyr	His	Leu	Lys	Gln	He	۸sn	Ser	Pro	Met	Met	Thr
	770					775					780				
Val	Ser	Ala	Tyr	Asn	Asn	Phe	Leu	Asn	Tyr	Val	Ser	Ser	Leu	Pro	Ser
785					790					795					800
Thr	Lys	Ala	Gly	Pro	Lys	Ile	Val	Ser	His	Leu	Leu	His	Leu	Val	Asp
	_		-	805	_				810					815	
Asn	Lvs	Glu	Ser		Glu	Asn	He	Ser		Asn	Asp	Asp	Tvr	Leu	Lvs
11011	250	0.0	820					825					830		-, -
U:c	C1-	D-0	Glu	Ho	Sor	Ι	Cln		Cln	Lou	Lou	Ara		Lou	Trn
1115	GIII		Giu	116	261	Leu		mer	GIII	Leu	LEU		Gly	Leu	11 þ
		835		٥.		m	840					845			
Gln		Cys	Pro	GIn	Ala	-	Phe	Ser	Met	Val		Glu	His	Leu	Leu
	850					855					860				
Val	Leu	Ala	Leu	Lys	Thr	Ala	Tyr	Gln	Ser	Asn	Thr	Val	Ala	Ala	Cys
865					870					875					880
Ser	Pro	Phe	Val	Leu	Gln	Phe	Leu	Gln	Gly	Arg	Thr	Leu	Thr	Leu	Gly
				885					890					895	
Ala	Leu	Asn	Leu	Gln	Tyr	Phe	Phe	Asp	His	Pro	Glu	Ser	Leu	Ser	Leu
			900					905					910		
Leu	Arg	Ser	He	His	Phe	Pro	He	Arg	Glv	Asn	Lvs	Thr	Ser	Pro	Arg
	8	915					920	6	5		-,-	925			6
A1 a	Hic		Ser	Val	Lau	Glu		Cue	Phe	Asn	Lve		Gln	Val	Pro
піа		THE	Jei	Val	Leu	935	1111	Cys	THE	лэр	940	JCI	0111	vai	110
Th	930		٥,		т		c	4.1	Di	C 1		и.		C 1	т
	He	Asp	Gln	Asp	-	Ala	Ser	Ala	Phe		Pro	мет	Asn	GIU	
945					950		_			955	_	_			960
Glu	Arg	Asn	Leu	Ala	Glu	Lys	Glu	Asp	Asn	Val	Lys.	Ser	Tyr	Met	Asp
				965					970					975	
Met	Gln	Arg	Arg	Ala	Ser	Pro	Asp	Leu	Ser	Thr	Gly	Tyr	Trp	Lys	Leu
			980					985					990		
Ser	Pro	Lys	Gln	Tyr	Lys	He	Pro	Cys	Leu	Glu	Val	Asp	Val	Asn	Asp
		995			-		1000	•				1005			
He	Asn	Val	Val	Glv	Gln	Asp	Met	Leu	Glu	He	Leu	Met	Thr	Val	Phe
	1010			0.5		1015		200	012		1020				
		Sor	Gln	1.00			Lou	Hic	Lou			Sor	Ara	Cly	Dho
	міа	361	(3111	_		Giu	LEU	1115			1112	GEI	лгв		
1025					1030					1035			-		1040
He	Glu	Ser	He		Pro	Ala	Leu			Ser	Lys	Ala			lhr
				1045					1050					1055	_
Lys	Cys	Ser	He	Ser	Lys	Leu	Glu	Leu	Ser	Ala	Ala	Glu	Gln	Glu	Leu
			1060					1065					1070		
Leu	Leu	Thr	Leu	Pro	Ser	Leu	Glu	Ser	Leu	Glu	Val	Ser	Gly	Thr	Ile
		1075					1080					1085			
Gln	Ser	Cln	Asn	Clh	Πρ	Phe	Pro	Acn	Len	Asn	Lvs	Phe	Leu	Cvs	Leu

配列番号: 4 配列の長さ: 1295 配列の型: アミノ酸 26

1090		1095	1	100
Lys Glu Leu	Ser Val	Asp Leu Glu	Gly Asn Ile	Asn Val Phe Ser Val
1105	1	110	1115	1120
Ile Pro Glu	Glu Phe I	Pro Asn Phe	His His Met	Glu Lys Leu Leu Ile
	1125		1130	1135
Gln Ile Ser	Ala Glu 1	Tyr Asp Pro	Ser Lys Leu	Val Lys Leu Ile Gln
	1140		1145	1150
Asn Ser Pro	Asn Leu 1	His Val Phe	His Leu Lys	Cys Asn Phe Phe Ser
1155		1160	J	1165
	Ser Leu l		Leu Val Ser	Cys Lys Lys Leu Thr
1170		1175		.180
	Phe Ser			Val Pro Phe Val Ala
1185		190	1195	1200
				Asn Leu Glu Gly Gln
Ser Bea 110	1205	ric ocr bea	1210	1215
Cin Pho Pro		Clu Thr Sor		Ala Tyr Ile Leu Gly
	1220		1225	1230
	ASII Leu		He Leu Flo	Thr Gly Asp Gly Ile 1245
1235	A1 1	1240	C1 C1 C	-
-	Ala Lys			Gln Gln Leu His Cys
1250		1255		260
_				Asp Asp Ser Val Val
1265		270	1275	1280
Glu lle Ala	•	Ala lle Ser	-	Gln Lys Leu Glu Asn
	1285		1290	1295
Leu Lys Leu			He Thr Glu	
				Glu Gly Tyr Arg Asn
	1300		1305	1310
Phe Phe Gln	1300	Asp Asn Met	1305	1310 Gln Glu Leu Asp Ile
Phe Phe Gln 1315	1300 Ala Leu .	Asp Asn Met 1320	1305 Pro Asn Leu	1310 Gln Glu Leu Asp Ile 1325
Phe Phe Gln 1315 Ser Arg His	1300 Ala Leu .	Asp Asn Met 1320 Glu Cys Ile	1305 Pro Asn Leu	1310 Gln Glu Leu Asp Ile
Phe Phe Gln 1315 Ser Arg His 1330	1300 Ala Leu Phe Thr	Asp Asn Met 1320 Glu Cys Ile 1335	1305 Pro Asn Leu Lys Ala Gln	1310 Gln Glu Leu Asp Ile 1325 Ala Thr Thr Val Lys 1340
Phe Phe Gln 1315 Ser Arg His 1330	1300 Ala Leu Phe Thr	Asp Asn Met 1320 Glu Cys Ile 1335	1305 Pro Asn Leu Lys Ala Gln	1310 Gln Glu Leu Asp Ile 1325 Ala Thr Thr Val Lys
Phe Phe Gln 1315 Ser Arg His 1330	1300 Ala Leu Phe Thr	Asp Asn Met 1320 Glu Cys Ile 1335	1305 Pro Asn Leu Lys Ala Gln	1310 Gln Glu Leu Asp Ile 1325 Ala Thr Thr Val Lys 1340
Phe Phe GIn 1315 Ser Arg His 1330 Ser Leu Ser 1345	Ala Leu . Phe Thr Gln Cys .	Asp Asn Met 1320 Glu Cys Ile 1335 Val Leu Arg 350	1305 Pro Asn Leu Lys Ala Gln Leu Pro Arg 1355	1310 Gln Glu Leu Asp Ile 1325 Ala Thr Thr Val Lys 1340 Leu Ile Arg Leu Asn
Phe Phe Gln 1315 Ser Arg His 1330 Ser Leu Ser 1345 Met Leu Ser	Ala Leu . Phe Thr Gin Cys . Trp Leu . 1365	Asp Asn Met 1320 Glu Cys Ile 1335 Val Leu Arg 350 Leu Asp Ala	1305 Pro Asn Leu Lys Ala Gln Leu Pro Arg 1355 Asp Asp Ile 1370	1310 Gln Glu Leu Asp Ile 1325 Ala Thr Thr Val Lys 1340 Leu Ile Arg Leu Asn 1360 Ala Leu Leu Asn Val 1375
Phe Phe Gln 1315 Ser Arg His 1330 Ser Leu Ser 1345 Met Leu Ser	Ala Leu . Phe Thr Gin Cys . Trp Leu . 1365	Asp Asn Met 1320 Glu Cys Ile 1335 Val Leu Arg 350 Leu Asp Ala	1305 Pro Asn Leu Lys Ala Gln Leu Pro Arg 1355 Asp Asp Ile 1370	1310 Gln Glu Leu Asp Ile 1325 Ala Thr Thr Val Lys 1340 Leu Ile Arg Leu Asn 1360 Ala Leu Leu Asn Val
Phe Phe Gln 1315 Ser Arg His 1330 Ser Leu Ser 1345 Met Leu Ser Met Lys Glu	Ala Leu . Phe Thr Gin Cys . Trp Leu . 1365	Asp Asn Met 1320 Glu Cys Ile 1335 Val Leu Arg 350 Leu Asp Ala Pro Gln Ser	1305 Pro Asn Leu Lys Ala Gln Leu Pro Arg 1355 Asp Asp Ile 1370	1310 Gln Glu Leu Asp Ile 1325 Ala Thr Thr Val Lys 1340 Leu Ile Arg Leu Asn 1360 Ala Leu Leu Asn Val 1375
Phe Phe Gln 1315 Ser Arg His 1330 Ser Leu Ser 1345 Met Leu Ser Met Lys Glu	Ala Leu Phe Thr Gln Cys 1 Trp Leu 1365 Arg His	Asp Asn Met 1320 Glu Cys Ile 1335 Val Leu Arg 350 Leu Asp Ala Pro Gln Ser	Pro Asn Leu Lys Ala Gln Leu Pro Arg 1355 Asp Asp Ile 1370 Lys Tyr Leu	1310 Gln Glu Leu Asp Ile 1325 Ala Thr Thr Val Lys 340 Leu Ile Arg Leu Asn 1360 Ala Leu Leu Asn Val 1375 Thr Ile Leu Gln Lys
Phe Phe Gln 1315 Ser Arg His 1330 Ser Leu Ser 1345 Met Leu Ser Met Lys Glu	Phe Thr Gln Cys Trp Leu 1365 Arg His 1380 Pro Phe	Asp Asn Met 1320 Glu Cys Ile 1335 Val Leu Arg 350 Leu Asp Ala Pro Gln Ser	1305 Pro Asn Leu Lys Ala Gln Leu Pro Arg 1355 Asp Asp Ile 1370 Lys Tyr Leu 1385	1310 Gln Glu Leu Asp Ile 1325 Ala Thr Thr Val Lys 340 Leu Ile Arg Leu Asn 1360 Ala Leu Leu Asn Val 1375 Thr Ile Leu Gln Lys
Phe Phe Gin 1315 Ser Arg His 1330 Ser Leu Ser 1345 Met Leu Ser Met Lys Giu	Phe Thr Gln Cys Trp Leu 1365 Arg His 1380 Pro Phe	Asp Asn Met 1320 Glu Cys Ile 1335 Val Leu Arg 350 Leu Asp Ala Pro Gln Ser	Lys Ala Gln Leu Pro Arg 1355 Asp Asp Ile 1370 Lys Tyr Leu 1385 Ile Gln Lys 1403	1310 Gln Glu Leu Asp Ile 1325 Ala Thr Thr Val Lys 340 Leu Ile Arg Leu Asn 1360 Ala Leu Leu Asn Val 1375 Thr Ile Leu Gln Lys
Phe Phe Gin 1315 Ser Arg His 1330 Ser Leu Ser 1345 Met Leu Ser Met Lys Giu	Phe Thr Gln Cys Trp Leu 1365 Arg His 1380 Pro Phe	Asp Asn Met 1320 Glu Cys Ile 1335 Val Leu Arg 350 Leu Asp Ala Pro Gln Ser	Lys Ala Gln Leu Pro Arg 1355 Asp Asp Ile 1370 Lys Tyr Leu 1385 Ile Gln Lys 1403	1310 Gln Glu Leu Asp Ile 1325 Ala Thr Thr Val Lys 1340 Leu Ile Arg Leu Asn 1360 Ala Leu Leu Asn Val 1375 Thr Ile Leu Gln Lys 1390
Phe Phe Gin 1315 Ser Arg His 1330 Ser Leu Ser 1345 Met Leu Ser Met Lys Giu	Phe Thr Gln Cys Trp Leu 1365 Arg His 1380 Pro Phe	Asp Asn Met 1320 Glu Cys Ile 1335 Val Leu Arg 350 Leu Asp Ala Pro Gln Ser	Lys Ala Gln Leu Pro Arg 1355 Asp Asp Ile 1370 Lys Tyr Leu 1385 Ile Gln Lys 1403	1310 Gln Glu Leu Asp Ile 1325 Ala Thr Thr Val Lys 1340 Leu Ile Arg Leu Asn 1360 Ala Leu Leu Asn Val 1375 Thr Ile Leu Gln Lys 1390
Phe Phe Gin 1315 Ser Arg His 1330 Ser Leu Ser 1345 Met Leu Ser Met Lys Giu	Phe Thr Gln Cys Trp Leu 1365 Arg His 1380 Pro Phe	Asp Asn Met 1320 Glu Cys Ile 1335 Val Leu Arg 350 Leu Asp Ala Pro Gln Ser	Lys Ala Gln Leu Pro Arg 1355 Asp Asp Ile 1370 Lys Tyr Leu 1385 Ile Gln Lys 1403	1310 Gln Glu Leu Asp Ile 1325 Ala Thr Thr Val Lys 1340 Leu Ile Arg Leu Asn 1360 Ala Leu Leu Asn Val 1375 Thr Ile Leu Gln Lys 1390
Phe Phe Gin 1315 Ser Arg His 1330 Ser Leu Ser 1345 Met Leu Ser Met Lys Glu Trp Ile Leu 1395	Phe Thr Gln Cys Trp Leu 1365 Arg His 1380 Pro Phe	Asp Asm Met 1320 Glu Cys Ile 1335 Val Leu Arg 350 Leu Asp Ala Pro Gln Ser Ser Pro Ile 1400	Table 1970 Asn Leu Lys Ala Gln Leu Pro Arg 1355 Asp Asp Ile 1370 Lys Tyr Leu 1385 Ile Gln Lys 1403 40 トポロジ	1310 Gln Glu Leu Asp Ile 1325 Ala Thr Thr Val Lys 1340 Leu Ile Arg Leu Asn 1360 Ala Leu Leu Asn Val 1375 Thr Ile Leu Gln Lys 1390
Phe Phe Gin 1315 Ser Arg His 1330 Ser Leu Ser 1345 Met Leu Ser Met Lys Glu Trp Ile Leu 1395	Phe Thr Gln Cys Trp Leu 1365 Arg His 1380 Pro Phe	Asp Asm Met 1320 Glu Cys Ile 1335 Val Leu Arg 350 Leu Asp Ala Pro Gln Ser Ser Pro Ile 1400	Table 1970 Asn Leu Lys Ala Gln Leu Pro Arg 1355 Asp Asp Ile 1370 Lys Tyr Leu 1385 Ile Gln Lys 1403 40 トポロジ	1310 Gln Glu Leu Asp Ile 1325 Ala Thr Thr Val Lys 1340 Leu Ile Arg Leu Asn 1360 Ala Leu Leu Asn Val 1375 Thr Ile Leu Gln Lys 1390 - : 直鎖状
Phe Phe Gin 1315 Ser Arg His 1330 Ser Leu Ser 1345 Met Leu Ser Met Lys Giu Trp Ile Leu 1395	Ala Leu Phe Thr Gln Cys 1 Trp Leu 1365 Arg His 1380 Pro Phe	Asp Asm Met 1320 Glu Cys Ile 1335 Val Leu Arg 350 Leu Asp Ala Pro Gln Ser Ser Pro Ile 1400	1305 Pro Asn Leu Lys Ala Gln Leu Pro Arg 1355 Asp Asp Ile 1370 Lys Tyr Leu 1385 Ile Gln Lys 1403 40 トポロジ 配列の和	1310 Gln Glu Leu Asp Ile 1325 Ala Thr Thr Val Lys 1340 Leu Ile Arg Leu Asn 1360 Ala Leu Leu Asn Val 1375 Thr Ile Leu Gln Lys 1390 一:直鎖状 類:タンパク質

Gln Leu Ala Lys Glu Leu Glu Glu Glu Glu Gln Lys Glu Arg Ala Lys . $35 \hspace{1.5cm} 40 \hspace{1.5cm} 45$ Met Gln Lys Gly Tyr Asn Ser Gln Met Arg Ser Glu Ala Lys Arg Leu

	50					55					60				
Lys 65	Thr	Phe	Val	Thr	Tyr 70	Glu	Pro	Tyr	Ser	Ser 75	Trp	Ile	Pro	Gln	G1u 80
Met	Ala	Ala	Ala	Gly 85	Phe	Tyr	Phe	Thr	G1y 90	Val	Lys	Ser	Gly	I 1e 95	Gln
Cys	Phe	Cys	Cys 100	Ser	Leu	He	Leu	Phe 105	Gly	Ala	Gly	Leu	Thr 110	Arg	Leu
Pro	Ile	Glu 115	Asp	His	Lys	Arg	Phe 120	His	Pro	Asp	Cys	Gly 125	Phe	Leu	Leu
۸sn	Lys 130	Лѕр	Val	Gly	Λsn	11e 135	Λla	Lys	Tyr	Asp	I le 140	۸rg	Val	Lys	۸sn
Leu 145	Lys	Ser	Arg	Leu	Arg 150	Gly	Gly	Lys	Met	Arg 155	Tyr	Gln	Glu	Glu	G1u 160
Ala	Arg	Leu	Ala	Ser 165	Phe	Arg	Asn	Trp	Pro 170	Phe	Tyr	Val	Gln	Gly 175	He
Ser	Pro	Cys	Val 180	Leu	Ser	Glu	Ala	Gly 185	Phe	Val	Phe	Thr	Gly 190	Lys	Gln
Asp	Thr	Val 195	Gln	Cys	Phe	Ser	Cys 200	Gly	Gly	Cys	Leu	Gly 205	Asn	Trp	Glu
Glu	G1y 210	Asp	Asp	Pro	Trp	Lys 215	Glu	His	Ala	Lys	Trp 220	Phe	Pro	Lys	Cys
G1u 225	Phe	Leu	Arg	Ser	Lys 230	Lys	Ser	Ser	Glu	G1u 235	Ile	Thr	Gln	Tyr	Ile 240
Gln	Ser	Tyr	Lys	Gly 245	Phe	Val	Asp	Ile	Thr 250	Gly	Glu	His	Phe	Val 255	Asn
Ser	Trp	Val	G1n 260	Arg	Glu	Leu	Pro	Met 265	Ala	Ser	Ala	Tyr	Cys 270	Asn	Asp
Ser	Ile	Phe 275	Ala	Tyr	Glu	Glu	Leu 280	Arg	Leu	Asp	Ser	Phe 285	Lys	Asp	Trp
Pro	Arg 290	Glu	Ser	Ala	Val	G1y 295	Val	Ala	Ala	Leu	Ala 300	Lys	Ala	Gly	Leu
Phe 305	Tyr	Thr	Gly	Ile	Lys 310	Asp	Ile	Val	Gln	Cys 315	Phe	Ser	Cys	Gly	Gly 320
Cys	Leu	Glu	Lys	Trp 325	Gln	Glu	Gly	Asp	Asp 330	Pro	Leu	Asp	Asp	His 335	Thr
Arg	Cys	Phe	Pro 340	Asn	Cys	Pro	Phe	Leu 345	Gln	Asn	Met	Lys	Ser 350	Ser	Ala
Glu	Val	Thr 355	Pro	Asp	Leu	Gln	Ser 360	Arg	Gly	Glu	Leu	Cys 365	Glu	Leu	Leu
	370		Ser			375					380				
I1e 385	Val	Pro	Glu	Met	Ala 390	Gln	Gly	Glu	Ala	G1n 395	Trp	Phe	Gln	Glu	Ala 400
Lys	Asn	Leu	Asn	G1u 405	Gln	Leu	Arg	Ala	Ala 410	Tyr	Thr	Ser	Ala	Ser 415	Phe
Arg	His	Met	Ser 420	Leu	Leu	Asp	Ile	Ser 425	Ser	Asp	Leu	Ala	Thr 430	Asp	His
Leu	Leu	Gly 435	Cys	Asp	Leu	Ser	Ile 440	Ala	Ser	Lys	His	Ile 445	Ser	Lys	Pro
Val	Cln	Glu	Pro	Len	Val	Len	Pro	Clu	Va1	Phe	Glv	Asn	Leu	Asn	Ser

	450					455					460				
Val	Met	Cys	Val	Glu	Gly	Glu	Λla	Gly	Ser	Gly	Lys	Thr	Val	Leu	Leu
465					470					475					480
Lys	Lys	He	Ala	Phe 485	Leu	Trp	Ala	Ser	G1y 490	Cys	Cys	Pro	Leu	Leu 495	Asn
Arg	Phe	G1n	Leu 500	Val	Phe	Tyr	Leu	Ser 505	Leu	Ser	Ser	Thr	Arg 510	Pro	Asp
Glu	Gly	Leu 515	Ala	Ser	Ile	Ile	Cys 520	Asp	Gln	Leu	Leu	Glu 525	Lys	Glu	G1y
Ser	Val 530	Thr	Glu	Met	Cys	Met 535	Λrg	Asn	Ile	Ile	G1n 540	Gln	Leu	Lys	Asn
G1n 545	Val	Leu	Phe	Leu	Leu 550	Asp	Asp	Tyr	Lys	G1u 555	Ile	Cys	Ser	He	Pro 560
G1n	Val	Ile	Gly	Lys 565	Leu	Ile	Gln	Lys	Asn 570	His	Leu	Ser	Arg	Thr 575	Cys
Leu	Leu	Ile	Ala 580	Val	Arg	Thr	Asn	Arg 585	Ala	Arg	Asp	Ile	Arg 590	Arg	Tyr
Leu	Glu	Thr 595	Ile	Leu	G1u	He	Lys 600	Ala	Phe	Pro	Phe	Tyr 605	Asn	Thr	Val
Cys	I1e 610	Leu	Arg	Lys	Leu	Phe 615	Ser	His	Asn	Met	Thr 620	Arg	Leu	Arg	Lys
Phe 625	Met	Val	Tyr	Phe	G1y 630	Lys	Asn	Gln	Ser	Leu 635	Gln	Lys	Ile	Gln	Lys 640
Thr	Pro	Leu	Phe	Val 645	Ala	Ala	He	Cys	Ala 650	His	Trp	Phe	Gln	Tyr 655	Pro
Phe	Asp	Pro	Ser 660	Phe	Asp	Asp	Val	Ala 665	Val	Phe	Lys	Ser	Tyr 670	Met	G1u
Arg	Leu	Ser 675	Leu	Arg	Åsn	Lys	Ala 680	Thr	Ala	Glu	Ile	Leu 685	Lys	Ala	Thr
Val	Ser 690	Ser	Cys	Gly	Glu	Leu 695	Ala	Leu	Lys	Gly	Phe 700	Phe	Ser	Cys	Cys
Phe 705	Glu	Phe	Asn	Asp	Asp 710	Asp	Leu	Ala	Glu	Ala 715	Gly	Val	Asp	Glu	Asp 720
Glu	Asp	Leu	Thr	Met 725	Cys	Leu	Met	Ser	Lys 730	Phe	Thr	Ala	G1n	Arg 735	Leu
Arg	Pro	Phe	Tyr 740	Arg	Phe	Leu	Ser	Pro 745	Ala	Phe	Gln	Glu	Phe 750	Leu	Ala
Gly	Met	Arg 755	Leu	Ile	Glu	Leu	Leu 760	Asp	Ser	Asp	Arg	G1n 765	Glu	His	Gln
Asp	Leu 770	Gly	Leu	Tyr	His	Leu 775	Lys	Gln	Ile	Asn	Ser 780	Pro	Met	Met	Thr
Val 785	Ser	Ala	Tyr	Asn	Asn 790	Phe	Leu	Asn	Туг	Val 795	Ser	Ser	Leu	Pro	Ser 800
	Lys			805					810					815	
	Lys		820					825					830		
His	Gln	Pro 835	Glu	Ile	Ser	Leu	840					845			
Cı	Т 1	0	D	C 1	A 1 -	T	DL.	C	Max	Vo.1	C	C1	Uio	Ī	1

	31														32
	850					855					860				
Val		Λla	Leu	Lvs	Thr		Tyr	Gln	Ser	۸sn		Val	۸la	Лlа	Cys
865				- 7 -	870		,			875					880
Ser	Pro	Phe	Val	Leu 885	Gln	Phe	Leu	G1n	G1y 890	Arg	Thr	Leu	Thr	Leu 895	Gly
Ala	Leu	Asn	Leu 900		Tyr	Phe	Phe	Asp 905		Pro	G1u	Ser	Leu 910	Ser	Leu
Leu	Arg	Ser 915		His	Phe	Pro	I le 920		Gly	Asn	Lys	Thr 925		Pro	Arg
λla	Hic		Sor	Val	Lou	Clu	Thr	Cvs	Phe	۸sn	Ivs		Gln	Val	Pro
NIA	930	THE	361	vai	LCu	935	1111	Cys	THE	пор	940	JCI	G	,41	110
Thr		Asp	Gln	Asp	Tvr		Ser	Ala	Phe	Glu		Met	Asn	Glu	Trp
945			•		950				_	955					960
	Arg	Asn	Leu	Ala	Glu	Lys	Glu	Asp	Asn	Val	Lys	Ser	Tyr	Met	Asp
				965					970					975	
Met	Gln	Arg	Arg 980	Ala	Ser	Pro	Asp	Leu 985	Ser	Thr	Gly	Tyr	Trp 990	Lys	Leu
Ser	Pro	Lys	Gln	Tyr	Lys	Ile	Pro	Cys	Leu	Glu	Val	Asp	Val	Asn	Asp
		995					1000					1005			
	-	Val	Val	Gly			Met	Leu	Glu			Met	Thr	Val	Phe
	1010	_				1015		** .			1020				D.
	Ala	Ser	Gln	_		Glu	Leu	His			His	Ser	Arg		Phe
1025	C 2	C	т.		1030	4.1		C1		1035	T	41 -	C		1040
He	Glu	Ser		Arg 1045	Pro	Ala	Leu		Leu 1050	ser	Lys	Ala		va 1 1055	Inr
Lve	Cue	Sor			lue	Leu	Glu			Δla	Δla	Clu			Leu
ьуз	Uys		1060	JCI	Lys	bcu		1065	SCI	MIG	AIG		1070	oru	Deu
Leu	Leu			Pro	Ser	Leu			Leu	Glu	Val			Thr	He
		1075					1080					1085	,		
Gln	Ser	Gln	Asp	Gln	Ile	Phe	Pro	Asn	Leu	Asp	Lys	Phe	Leu	Cys	Leu
	1090					1095					1100				
Lys	Glu	Leu	Ser			Leu	Glu	Gly	Asn	He	Asn	Val	Phe	Ser	Val
1105					1110					1115	_	_	_		1120
He	Pro	Glu			Pro	Asn	Phe			Met	Glu	Lys			He
C1-	T 1 -	C		1125	т	A	D		1130	I	Vo.1	Luc		1135	C1-
Gin	116		ила 1140	Giu	Tyr	ASP		3er 1145	Lys	Leu	vai		1150	He	Gln
Acn	Sor			Leu	Hic	Val			Leu	Īve	Cvs			Phe	Ser
ASII		1155	71311	LCu	1113		1160	1113	Deu	by 3		1165	1110		bei
Asp			Ser	Leu	Met			Leu	Val	Ser			Lys	Leu	Thr
•	1170	5				1175					1180	,	,		
Glu	He	Lys	Phe	Ser	Asp	Ser	Phe	Phe	Gln	Ala	Val	Pro	Phe	Val	Ala
1185					1190					1195					1200
Ser	Leu	Pro	Asn	Phe	He	Ser	Leu	Lys	He	Leu	Asn	Leu	Glu	Gly	Gln
				1205					1210					1215	
Gln	Phe	Pro	Asp	Glu	Glu	Thr	Ser	Glu	Lys	Phe	Ala			Leu	Gly
_			1220		_	_		1225	_		m-		1230	a -	
Ser		_		Leu	Glu			He	Leu	Pro			Asp	Gly	He
т		1235		T	Ι		1240	Cı	C1	C		1245	I	U:-	C
ıyr	arg	vai	ата	Lys	Leu	пе	116	GIN	GIN	UyS	GIII	GIII	ı.eu	1115	Cys

1250 1255 1260

Leu Λrg Val Leu Ser Phe Phe Lys Thr Leu Λsn Λsp Λsp Ser Val Val
265 1270 1275 1280

Glu Ile Gly Glu Leu Val Phe Gln Leu Ala Trp Lys Pro Val Val
1285 1290 1295

【図面の簡単な説明】

【図1】卵巣内における卵胞の発達過程を示した模式図である。

【図2】ハイブリダイゼーション用プローブの遺伝子位 10 置を示した模式図である。

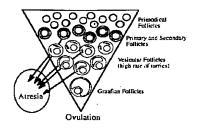
【図3】卵巣におけるNAIP遺伝子の発現を調べたin situ hybridization の結果であり、(A) はセンスリボプローブを用いた場合、(B) はアンチセンスリボプローブを用いた場合を示す。

【図4】マウスNAIP遺伝子のノーザンブロット分析

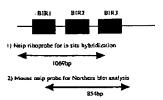
の結果である。(A) はマウス各組織での遺伝子発現を示し、各レーンは、1:精巣、2:腎臓、3:骨格筋、4:肝臓、5:肺、6:脾臓、7:脳、8:心臓である。(B) はマウスの発達過程における卵巣内での遺伝子発現を示し、各レーンは、1:2日令、2:3週令、3:12週令、4:分娩3日目、5:18週令である。

【図 5 】卵巣内におけるNAIP遺伝子の発現が顆粒膜細胞に局在していることを示したin situ hybridizatio n (上段、中断) およびTUNEL法 (下段) の結果である。

【図1】



【図2】



【図3】

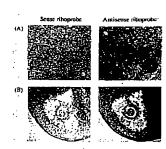
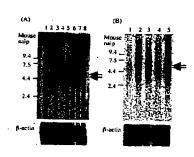
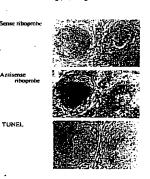


Fig. 1. Follicular development in the mouse ovary

【図4】



【図5】



フロントページの続き

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